1985

Hydrolysis and availability to plants of polyphosphates added to soils

Richard Peter Dick
Iowa State University

Dick, Richard Peter

HYDROLYSIS AND AVAILABILITY TO PLANTS OF POLYPHOSPHATES ADDED TO SOILS

Iowa State University PH.D. 1985

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages __✓__
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

_____

_____

Hydrolysis and availability to plants of polyphosphates added to soils

by

Richard Peter Dick

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Soil Fertility

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1985

TABLE OF CONTENTS

INTRODUCTION

Phosphorus (P) is an essential element for plant growth and is important in energy transfer in all living organisms. All soils contain P, but the amounts present and availability to plants vary widely among soils. Phosphorus is present in soils as inorganic compounds, which have very low solubilities, and as organic compounds that contain esters of phosphoric acid, which is slowly mineralized by enzyme-mediated reactions.

To correct for the low plant availability of P in soils, man has applied various P-containing materials to soils. Since the 19th century, the use of P fertilizers has increased dramatically with the advent of ordinary superphosphoric acid, which is produced by reacting rock phosphate with sulfuric acid. However, when P is added to soils, a large portion of the P is rendered unavailable to plants due to fixation or sorption reactions with soil constituents, which results in only 10-30% of the P added to soils being recovered by plants.

To overcome this P fixation problem, alternatives to the conventional P fertilizer (orthophosphate compounds) have been tested. Some organic P compounds added to calcareous soils have shown greater soil infiltration than orthophosphate compounds. Specific condensed inorganic phosphates (polyphosphates) have characteristics that could be useful in improving P fertilizer use efficiency. Trimetaphosphate, a cyclic polyphosphate, is unique among polyphosphates in that it remains

soluble in the presence of metal ions, such as Mg^{2+}, Ca^{2+}, and Ba^{2+}, and is not sorbed by soil constituents. Linear oligomers added to soils may have residual effects by slowly releasing orthophosphate over the growing season.

The key to the availability of polyphosphate-P is the rate of hydrolysis which is mediated chemically and biochemically by a group of enzymes known as phosphatases. Because plants largely take up P in the orthophosphate form, compounds such as polyphosphate must first undergo a hydrolysis reaction in order to release orthophosphate for absorption by plant roots. However, the degradation of polyphosphates should not proceed too rapidly, otherwise, the favorable characteristics of polyphosphates would be quickly lost, thus diminishing their potential for increasing P efficiency. With the exception of pyrophosphate, little information is available about hydrolysis of polyphosphates in plant root-soil systems.

Plant roots have the ability to hydrolyze certain P compounds. This is an important feature because roots that could hydrolyze a polyphosphate would not have to rely solely on hydrolysis reactions in soils in order to make polyphosphate-P available for plant uptake. To distinguish between the role of roots and rhizoplane microorganisms, the hydrolysis rates of a polyphosphate in the presence of sterile and nonsterile intact roots can be compared.

The hydrolysis of P compounds added to soils is intimately

associated with the mineralization of organic matter in soils as a whole. However, using current methods, the hydrolysis of P compounds added to soils can be studied. Furthermore, the relative contribution of biochemical reactions (phosphatases) and chemical reactions (e.g., metal ion-mediated reactions) towards the hydrolysis of P compounds in soils can be determined. Hydrolysis reactions are temperature dependent; studies have shown that hydrolysis of pyrophosphate is inhibited in soils under cool temperatures. Thus, temperature is an important factor to consider in determining the availability of polyphosphate-P for plants during cool growing periods.

The degradation of polyphosphates added to soils is the result of complex interactions of several factors. These include: cations, colloidal gels, pH, and enzymes. Conversely, sorption reactions of linear oligomers with soil constituents could limit the availability of these compounds to hydrolytic reactions. By using statistical procedures (factor analysis), favorable characteristics of a P fertilizer could be identified, which could set priorities for the development of model P fertilizer compounds.

The individual hydrolytic reactions of plant roots and soils are important in understanding the degradation process of polyphosphates in plant-root-soil systems. However, to assess the availability of orthophosphate released from polyphosphates it is necessary to measure the uptake of P by plants

growing in soils amended with polyphosphate. Investigations are needed that would compare the efficiency of P recovered by plants from polyphosphates of known structure with that of the conventional P fertilizer, orthophosphate.

Recently, some polyphosphates (e.g., long, linear oligomers) of known structure have become readily available. Therefore, this study was conducted on 8 linear oligomers (P_2, P_3, P_5, P_{15}, P_{25}, P_{35}, P_{45}, and P_{65}) and one cyclic polyphosphate (trimetaphosphate) with the following objectives: (1) to assess the hydrolysis of polyphosphates by corn roots, (2) to determine the degree of hydrolysis of polyphosphates added to soils, (3) to assess factors affecting hydrolysis of polyphosphates added to soils, and (4) to evaluate the potential of polyphosphates as sources of P for plants.

LITERATURE REVIEW

Phosphorus makes up about 0.12% of the earth's crust. It is present in all soils and rocks, in water, and in plant and animal remains. Phosphorus is indispensable for all forms of life because of its genetic role in ribonucleic acid (RNA) and function in energy transfer via adenosine triphosphate (ATP).

With the possible exception of nitrogen, no other element has been shown to be as critical for growth and development of plants as P. Although the amount of total P in an average mineral soil compares favorable with that of N, it is much lower than K, Ca, or Mg. The prevalence of Al, Fe and Ca, which link P in highly insoluble compounds in soils, ensures the low availability of P in natural ecosystems. In soil-plant-animal systems, less than 10% of the P in soils enters the plant-animal life cycle (Ozanne, 1980). There are three major P fractions in soils: organic P, which may account for up to 50% of the total P; insoluble inorganic fraction; and a very small, highly variable, soluble fraction that can be absorbed by plants.

When P fertilizers are added to soils to increase the concentration of P in the soil solution, not all the P remains in solution to be available for growing plants because of sorption and precipitation reactions with soil constituents. In acid soils amended with P fertilizers, P will precipitate

with soluble Fe, Al and Mn. Iron and Al reactions with orthophosphate probably result in the formation of hydroxy phosphates. Phosphorus will also react with insoluble Al and Fe hydrous oxides in acid soils. In alkaline soils, fertilizer P will react with Ca and its carbonate to form insoluble calcium phosphates. Initially, a considerable portion of the applied P is available to plants, but with time, the adsorbed and precipitated P compounds become more stable and less soluble in the solution phase (Barrow and Shaw, 1975). Applied P can be lost through leaching and erosion processes and be immobilized in the organic matter fraction of soils. However, these processes play a minor role in determining P availability to plants compared with the P fixation reactions.

A suggested alternative to the conventional fertilizer form, orthophosphate, which is fixed in soils, is the use of P compounds that are not sorbed by soils and that would remain soluble, allowing for penetration of P into the rooting zone. The organic P compounds such as glycerophosphate and methyl ester phosphate were tested by Rolston et al. (1975). They showed that these compounds remained soluble in soils, but hydrolysis reactions were quite rapid which limited the usefulness of these compounds as P fertilizers. Nonetheless, it would be desirable to find P compounds that are not sorbed by soils and that could remain available to plants throughout the growing season.

Characteristics of Polyphosphates

The first report of condensed inorganic phosphates dates back to 1816, when Berzelius found that the ignition of orthophosphoric acid formed a vitreous product which was able to precipitate proteins (Van Wazer, 1958). In 1833, Graham produced a condensed phosphate which he named metaphosphate (Graham, 1833). However, it was soon shown that this metaphosphate was a mixture of closely related compounds which differed mainly in their degree of polymerization (Kulaev, 1979). Because of the pioneering work of Thilo, Van Wazer, Samuelson, Ebel, and Boulle, the present knowledge of the chemical structures and properties of condensed phosphates (polyphosphates) have been established (see Kulaev, 1979).

Polyphosphates are generally characterized as pentavelent P compounds in which various numbers of tetrahedral PO_4^{3-} groups are linked together by oxygen bridges (Thilo, 1962). Condensed inorganic phosphates fall into three classes: (1) linear polyphosphates, (2) cyclic polyphosphates, and (3) ultraphosphates or complex cross-linked phosphates.

Linear condensed phosphates are unbranched structures with the elementary composition of $M_{n+2}\ P_nO_{3n+1}$. The series range from pyrophosphate with a chain length of two to the insoluble, crystalline Kurrol's and Maddrell's salts of chain length around 10^4. All other polyphosphates, including "metaphosphate" and Grahams salt must be regarded as mixtures contain-

ing various proportions of species of different molecular size and structure. The linear polyphosphates are alkali stable but acid labile. Typically, hydrolysis of polyphosphate to orthophosphate (Pi) by 1 N mineral acid requires about 15 minutes at 100°C (Thilo, 1962). Linear polyphosphates are nonspecific and will form complexes with most metallic cations (Corbridge, 1974).

Cyclic, condensed phosphates conform to the general elementary formula $M_n P_n O_{3n}$ and are designated metaphosphates. The tri- and tetrametaphosphates are well-known but synthetic metaphosphates with up to 8 P atoms per ring are possible (Kulaev, 1979). A strong alkali converts metaphosphates to their corresponding linear counterparts and are ultimately hydrolyzed to Pi when heated in acid (Thilo, 1965).

Cross-linked, condensed phosphates or "ultraphosphates" occur in certain melts. Their characteristic features are the presence of branching points and phosphate groups which share three oxygen atoms with the neighboring phosphate groups (Van Wazer, 1958). Branching points are readily hydrolyzed in water and, therefore, biological occurrence of ultraphosphates would not be expected (Harold, 1966).

Since polyphosphates contain P atoms in the fully oxidized state, the condensed phosphates (excluding ultraphosphates) are reasonably stable to chemical attack. The ultimate hydrolysis product is Pi, although the rate and route of hydroly-

sis varies both among polyphosphates and according to environmental conditions (Corbridge, 1974). The principal factors influencing the chemical hydrolysis rate of polyphosphates are usually temperature, pH, and concentration. The linear and cyclic metaphosphates are hydrolyzed extremely slow at neutral pH and room temperature. The half-life of P-O-P bonds in linear polyphosphates at pH 7 and 25°C is several years (Kulaev, 1979). Increasing temperature and the concentration of H ions, or the presence of cations and enzymes accelerate the hydrolysis rates of polyphosphates. Enzymatic hydrolysis can increase the hydrolysis rate as much as 10^6 (Shen and Morgan, 1973).

The degradation of polyphosphates proceeds in two ways (Thilo, 1965). The first type of degradation occurs by clipping of the end group of the chain to form Pi, which is of zero order with respect to the total polyphosphate. The second type of degradation becomes increasingly important as the chain length increases beyond the pentaphosphate stage. This mechanism involves hydrolytic scission in the interior of the chain which results in the formation of short chains or trimetaphosphate. This mechanism is a first-order reaction (Thilo, 1965).

A variety of analytical methods have been developed for analysis of polyphosphates. Harold (1966), in his review, outlined a number of methods for chemical estimation of polyphosphates. These included general fractionation schemes utiliz-

ing various extracts such as cold acids, or removal by precipitation or adsorption by charcoal. Paper chromatography permits resolution of a mixture of polyphosphates into broad classes according to average chain length (Ohashi and Van Wazer, 1964). Column chromatography can be used to separate polyphosphate species. Czech and Hrychyshyn (1966) developed a column chromatographic method utilizing an anion-exchange resin to separate the P species and an auto analyzer to quantify each species. Recently, use of ion chromatography and high pressure liquid chromatography for analysis of polyphosphates have been developed. Cytochemical detection of polyphosphates is possible by using strains or by precipitating in situ with lead (Wirda, 1959) or by identification of polyphosphate granules in electron micrographs (Drews, 1962). The cytological procedures are qualitative indexes for polyphosphate species.

Polyphosphates are widely distributed among bacteria, blue-green algae, fungi, protozoa, and algae. Metaphosphates and linear phosphates with chain length as long as 500 P units have been isolated in microbial tissue (Harold, 1963).

Polyphosphates in cells form complexes with proteins or nucleic acids because they are strong polyanions which precipitate and combine with positively charged macromolecules. The amounts of "soluble" and "insoluble" polyphosphates will therefore depend upon the availability of a basic receptor and,

thus, in some organisms, lack of receptor sites allows for accumulation of "soluble" polyphosphates (Kulaev, 1979). Polyphosphates have been identified in volutin granules of cells (Drews, 1962; Wirda, 1959).

The function of polyphosphate accumulation appears to be for P reserve and to regulate the P economy of the microbial cells. Harold (1966), in his review, discounted that polyphosphates are used directly as energy sources but rather they are used in a physiological role.

Polyphosphates accumulate in microbial populations of soils and environmental factors greatly influence the level of accumulation. Deposition of polyphosphates in cells is promoted during the stationary or plateau phase of normal growth or when metabolism is slowed due to nutrient deficiency or imbalance. Nonetheless, accumulation of polyphosphates is transitory in soils (Lawry and Jensen, 1979). Ghonsikar and Miller (1973) found that the addition of excess amounts of Pi to glucose-amended soils resulted in accumulation of large quantities of acid-labile polyphosphates (up to 22 μg P/g soil) after 14 days of incubation. Pepper et al. (1976) found that the amount of polyphosphate accumulated was directly related to the Pi concentrations added, up to the maximum of 1000 μg P/g soil. They found significant amounts of polyphosphate accumulation with additions of 100 to 200 μg P/g soil (levels approaching normal fertilizer rates). They also reported that

polyphosphate synthesis increased with decreasing solubility of phosphate compounds added to soils. This suggested that polyphosphate synthesis accompanies P solubilization in soils.

Phosphatase Activity of Plant Roots

The chemical and biological reactions that occur in the soil-plant-root interface are important not only in absorption of P but also in the mineralization of unavailable P forms. Plants have been shown to utilize some condensed phosphates or organic P compounds directly. Sutton and Larsen (1964) showed that labeled pyrophosphate could be taken up directly in water culture by barley (Hordeum vulgare). This was further confirmed by Gilliam (1970) who used labeled pyrophosphate and paper chromatography on root extracts to show that pyrophosphate could be taken up directly.

Although a few studies have documented that low-molecular-weight P compounds may be utilized directly, the absorption of P from complex P compounds is usually as Pi following hydrolysis by phosphatases of soils, roots, or rhizosphere microorganisms. Phosphatases are a group of enzymes known to catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). Two orthophosphoric monoester phosphohydrolase enzymes, acid phosphatase and alkaline phosphatase, have been studied extensively. These enzymes are so classified because their optimum activity is related to either

an acid or alkaline pH range. Both acid and alkaline phosphatases are widely distributed in plants, animal tissue, and soils (see review by Juma, 1976).

Several phosphatases in roots have been reported. These include ATP-ase (Chang and Bandurski, 1964; Shaykh and Roberts, 1976), acid phosphatase (Spencer, 1954; McLean and Gahan, 1970; Hall and Davie, 1971; Bieleski, 1971; Juma, 1976), alkaline phosphatase (Bieleski, 1974), and glycerophosphatase (Ratner and Samoilova, 1955; Hall and Butt, 1968; McLean and Gahan, 1970).

To hydrolyze compounds in the medium surrounding the root, the enzyme must be located exocellularly. To test whether or not phosphatases are released from plant roots into solutions, Chang and Bandurski (1964) added ATP or pyrophosphate substrate to a solution in the presence of sterile corn (Zea mays) roots and to the same solution after the sterile corn roots had been removed. Their findings suggested that ATP-ase and pyrophosphatase were not excreted into the media. The authors concluded that these enzymes are located exterior to the permeability barrier of root cells but still are tightly bound to the cell wall. Conversely, Juma (1976), in a similar study, found that acid phosphatase of sterile corn and soybean (Glycine max) roots were excreted into the surrounding medium.

Histochemical studies by Gahan and Maple (1966) revealed the presence of acid phosphatase activity (using Gomori medium)

in the exterior cells of the root cap (within a 2-4 minute incubation) and localization of this enzyme in particles in protoxylem cells during the early stages of differentiation in Vicia faba. They suggested that this enzyme activity may occur in lysome-like particles in these cells. Using similar techniques, Hall (1969) found intense β-glycerophosphatase activity in the cell walls of the outer cells of corn roots, endodermis, pericycle, and in the gelatinous wall between the root cap and epidermis with lesser activity in the cytoplasm in the outer root cap cells. Similar results were reported by Hall and Davie (1971) with highest β-glycerophosphate activity occurring at the root surface and at the particulate sites in the cytoplasm. Distribution of acid phosphatase, ATP-ase and glucose-6-phosphosphatase was most intense in the root cap, protoderm and cortex (Shaykh and Roberts, 1974). In other studies, Hall and Butt (1968) found a significant correlation between phosphatase activity of cell-wall suspensions of roots and phosphatase activity of intact roots. This was reflected in nearly identical Michaelis-Menten constants and optimal pH values for phosphatase activity of cell-wall suspension and intact roots. The results suggest that the exocellular phosphatase is associated with the cell wall. On a macro-morphological scale, Juma (1976) found higher acid phosphatase activity in root hairs than the root tips of corn. Also, phosphatase activity decreased markedly with distance from the tip

in soybeans, but corn roots did not show this decrease with distance.

One question that arises is whether phosphatase activity has any strict morphological role in structural differentiation. If this is the case, any mineralization of macro-molecular P compounds would only be incidental. McLean and Gahan (1970) observed discernible enzyme activity that was not related to morphological activity. They found that cells, morphologically identical, exhibited different responses to the same acid phosphatase substrate according to their location within the root; thus, suggesting that the root is specifically adapted for mineralization of macro-molecular P compounds at the rhizoplane. Further proof for this concept was provided by Bieleski (1971) who found that when ^{32}P labeled glucose-1-phosphate was exposed to Spirodela, ^{32}P labeled inorganic phosphate appeared in the external solution long before it appeared in the tissue itself.

Various methods have been developed to study the activity of root phosphatases. These can be broadly categorized into two groups: root homogenate studies and intact root studies. In these studies, the rates of hydrolysis of P compounds is determined after incubating the substrate in the presence of root homogenate or intact roots for a specified period and measuring the product of hydrolysis. The rates of hydrolysis of P compounds have most commonly been determined by measuring

the appearance of the product, Pi. The most widely used method for P determination are the procedures outlined by Martin and Doty (1949) and Murphy and Riley (1962). However, inorganic condensed phosphates or organic P compounds interfere with these methods. This can result in as much as 10% error if color development and subsequent spectrophotometric readings are done within 10 min after sampling. This error will increase further with longer color development periods because of hydrolysis of these P compounds by the acid reagents used in these procedures (Dick and Tabatabai, 1977b). The inaccuracy of these methods has, until recently, been overlooked and should be considered in interpreting results of studies where these methods were used to assay phosphatase activity. To overcome this problem, Dick and Tabatabai (1977b) developed a method for determination of Pi that involves a rapid formation of heteropoly blue color by reaction of Pi with molybdate ions in the presence of ascorbic acid and trichloroacetic acid and complexation of the excess molybdate ions with a citrate-arsenite reagent to prevent further formation of blue color. This method insures that no hydrolysis of P compounds takes place during determination of Pi and provides greater accuracy for assaying phosphatase activity in the presence of acid labile P compounds.

Root phosphatase activity has been studied extensively by use of root homogenate (Hall and Butt, 1968; Reid and Bieleski,

1970; Juma, 1976; Dick, 1980; and others). This approach is particularly useful for large experiments where many data points are collected, because the procedure is relatively simple and of short duration. It has been typically used to determine optimal pH, temperature effects, and rates of activity for root phosphatases. Hall and Butt (1968) showed that the activities of a root cell-wall suspension and intact roots are virtually identical and further showed identical relations with respect to pH, substrate concentrations, and competitive inhibition by molybdate and Pi ions. This study indicates results from cell-wall suspension studies should be representative of phosphatase activity in intact roots.

In order to study the role of phosphatases located exocellularly, the use of intact roots is preferable. To isolate the root enzymes, a method to eliminate rhizoplane organisms is required. Estermann and McLaren (1961) found that dipping roots in various sterilants (1% NaOCl, 60% ethanol, 1/10,000 Ag NO_3) for approximately 10 minutes (exact times varied with each solution) and rinsing with sterile water resulted in seedlings that remained sterile for one day. They acknowledged that root dipping is a rather unreliable method and that a preferable method is to sterilize the seeds first and then germinate the seeds on sterile growth media. The latter method has been used by numerous workers with such sterilants as NaOCl, H_2O_2, and ethanol (Estermann and McLaren, 1961;

Barber and Loughmann, 1967; Savant and Racz, 1972; Ridge and Rovira, 1971; Juma, 1976).

McGeorge (1939) reported that the presence of plant roots (nonsterile) greatly accelerated the rate of hydrolysis of metaphosphate. Sterile corn roots were shown by Rogers et al. (1940) to hydrolyze nucleic acid, nucleotides, and glycerol phosphates. Estermann and McLaren (1961) found that sterile barley and tomato (Lycopersicum esculentum) roots caused rapid hydrolysis of β-glycerolphosphate. Also, it is known that adenosine triphosphate, ribonucleic acid, and deoxyribonucleic acid are hydrolyzed by sterile corn roots (Chang and Bandurski, 1964). Organic P compounds tested by Juma (1976) had rates of hydrolysis in the following order: p-nitrophenyl > phenolphalein diphosphate > β-glycerophosphate > α-glycerophosphate (40%) and β-glycerophosphate >° glucose-6-phosphate > phosphocholine, with no hydrolysis of o-carboxyphenyl phosphate or lecithin by sterile corn or soybean roots.

Pyrophosphate has been the most extensively studied polyphosphate compound relative to root hydrolysis. According to Chang and Bandurski (1964), plant roots contain the pyrophosphatase enzyme which hydrolyzes pyrophosphate. Gilliam (1970) reported that 40 to 55% of the pyrophosphate in nonsterile wheat (Triticum sp.), corn and barley roots was hydrolyzed in a 24-h period. Pyrophosphate is hydrolyzed more rapidly than tripolyphosphate (Savant and Racz, 1972; Subbarao et al., 1977).

This probably occurs because tripolyphosphate is hydrolyzed stepwise with pyrophosphate being an intermediate to the final product of Pi. An exception to these findings is a study by Sutton and Larsen (1964) who found no hydrolysis of pyrophosphate by barley roots in a 2-h period. Gilliam (1970) suggested that the reason for these results was that Sutton and Larsen (1964) used plants adequately supplied with P. Gilliam (1970) noted that P-deficient roots of wheat have much greater phosphatase activity than nondeficient roots.

Root-solution studies have indicated that plant species vary in their rates of hydrolysis of P compounds. The rate of hydrolysis of pyrophosphate on a per unit root-weight basis has been shown to be roughly equal for wheat and barley, whereas corn is less efficient than wheat and barley (Gilliam, 1970). Savant and Racz (1972) found that nonsterile roots of wheat showed a greater rate of hydrolysis of pyrophosphate and tripolyphosphate than did pea (Pisum sp.). But under sterile conditions, pea and wheat roots showed nearly equal hydrolysis rates. Subbarao et al. (1977) found that nonsterile roots of corn hydrolyzed pyrophosphate and tripolyphosphate faster than soybean roots. Juma (1976) reported that sterile soybean roots had a somewhat higher rate of hydrolysis than sterile corn roots using p-nitrophenyl phosphate as the substrate.

Environmental factors such as pH and temperature will affect the rate of hydrolysis by enzymatic systems. Studying

the hydrolysis of pyrophosphate by barley roots, Sutton and Larsen (1964) reported an optimal pH of 5.0. Comparing the rate of hydrolysis of β-glycerophosphate by nonsterile cell suspensions of barley roots with that by intact root tips, Hall and Butt (1968) found slight differences in optimal pH values, 6.2 vs 6.1, respectively. Studies with intact wheat roots in the presence of disodium (4-nitrophenyl) phosphate showed the highest phosphatase activity at pH 4.5 (Ridge and Rovira, 1971). Using p-nitrophenyl phosphate as a substrate, Juma (1976) found that the optimal pH of acid phosphatase in corn- and soybean-root homogenate occurs at pH 4.0 and 5.0, respectively. Dick (1980) found maximum pyrophosphatase activity of corn-root homogenate to be pH 6.0. He also compared three buffers: citrate, acetate, and modified universal buffer, and concluded that the modified universal buffer was the most suitable for this type of assay. A complicating factor found by Estermann and McLaren (1961) is that barley roots in buffer released PO_4-P from the roots as pH decreased below pH 5.0. In spite of this, they estimated optimal pH of phosphatase activity by using β-glycerophosphate as the substrate to be 5.3. None of the other intact root studies included this type of control which should be considered in interpreting results. All of these studies reported no alkaline phosphatase activity. An exception to this was that reported by Bieleski (1974). He showed that P-deficient Spirodela had a bimodal

pH curve with a shoulder at pH 6.0 and an optimum pH of 7.5, whereas the control showed a single peak at pH 5.7.

These results of pH effect show that there is differential pH optima between plant species. They further suggest that there may be different pH optima for hydrolysis of various P compounds within a plant species. However, there is little data presently available to substantiate this latter statement because nearly all the studies reviewed used only one substrate.

Temperature has been shown to significantly affect phosphatase activity. Using β-glycerophosphate as the substrate, Rogers et al. (1940) found maximum phosphatase activity of roots to be 45°C, while Estermann and McLaren (1961) reported maximum activity of 38°C for barley roots using the same substrate. Juma (1976) reported an optimal temperature of 60°C for acid phosphatase in corn- and soybean-root homogenate using p-nitrophenyl phosphate as the substrate.

The interrelationship of roots and the microbial population that develops at and around the root surface plays an important role in plant nutrition. Any beneficial or toxic products of microorganisms at the root surface can directly affect the growth and welfare of plants. The term rhizoplane usually is defined as the external root surface and closely adhering particles of soil and debris (Clark, 1949). While the rhizosphere is referred to as the region of contact between root and soil that is affected by roots (Starkey, 1958).

Tinker (1980), in his review, estimated that the mean population density of microorganisms in the rhizosphere is 10 or more times greater than the bulk soil. He also reported on a study where 4 to 10% of a grass-root surface was covered by bacteria, with a further 3% area covered with fungal hyphae. Apparently, the higher microbial populations of the rhizosphere are partially a result of a "mucigel" layer that develops around the root. The mucigel is mainly a product of root exudates, breakdown products of epidermal cells, and bacterially produced substances (Tinker, 1980). This probably provides a conducive habitat for microbial growth, although there is no evidence for any direct nutritional benefits (Rovira and McDougall, 1967; Mosse, 1975).

The rhizosphere microorganisms do utilize other materials supplied by the root which are complex compounds of organic acids, amino acids, and sugars originating from soluble exudates, sloughed-off root cap parts, root hair residues, and abraded epidermal cells (Tinker, 1980). Studies comparing sterile and nonsterile roots indicate rhizosphere organisms alter the rate of P uptake. Gerretsen (1948) reported a significantly higher uptake of P under nonsterile conditions than under sterile conditions by oats (Avena sativa), mustard Brassica nigra), sunflower (Helianthus annus), and rape (Brassica rapa) with tricalcium phosphate and bone meal being the P source. It has been shown in solution culture that at

low P concentrations (>0.5 ppm P) microbes can immobilize P, evidenced by the fact that sterile roots have higher rates of uptake than nonsterile roots. But when the P concentrations are increased, there is no difference in the amount of P uptake (Barber and Loughman, 1967; Asanuma et al., 1978). Bowen and Rovira (1966, 1969) found that nonsterile roots absorbed more P than sterile roots and transported the largest fraction to the shoot. Barber and Rovira (1975) showed that this effect of higher uptake by nonsterile roots decreased with age.

The mechanism for these synergistic effects on P uptake by microbes is not clearly understood. There is evidence that nonsterile roots have shorter roots, shorter root hairs, and reduced density but higher production of intensely branched roots compared to sterile roots (Tinker, 1980). It is also thought that bacteria may initiate hormonal activity similar to gibberellins and auxins which could affect P uptake (Lynch, 1976).

Another important role for rhizoplane organisms is in the transformation of macro-molecular P compounds to available forms such as Pi. Greaves and Welby (1965) studied the breakdown of organic phosphates by microorganisms from the root region of perennial pasture grasses. They surveyed the microorganisms isolated from the root surface, rhizosphere soil, and nonrhizosphere soil of the pasture grasses to determine their ability to attack organic P compounds. The compounds used were phenolphthalein diphosphate, sodium phytate,

lechithin, sodium glycerophosphate, DNA, and RNA. Using a dilution plate technique, these workers showed a high incidence of microorganisms capable of attacking organic phosphates. The microorganisms found in the root region attacked phenolphthalein diphosphate, glycerophosphate, and sodium phytate more readily than lecithin and nucleic acids. Greaves and Welby (1965), however, could not make any definite statement concerning the relationship between microbial breakdown of soil organic phosphates and phosphorus nutrition of plants.

Studies have shown that there is increased phosphatase activity on root surfaces in the presence of microorganisms. This has been demonstrated with the following substrates: β-glycerolphosphate (Estermann and McLaren, 1961), pyrophosphate and tripolyphosphate (Savant and Racz, 1972). Contrary to these results, Ridgě and Rovira (1971) found no increased phosphatase activity in the presence of microorganisms on wheat roots by using p-nitrophenyl phosphate as the substrate.

Phosphatase Activity in Soils

Since plants take up P mainly in the Pi form, polyphosphates and native organic or inorganic P compounds must first be hydrolyzed to Pi before it is taken up by plants. The hydrolysis is mediated by both chemical and biochemical reactions. A group of enzymes, collectively known as phosphatases, play an important role in the biochemical decomposition of

organic P compounds and the transformation of inorganic P compounds in soils (Kiss et al., 1975).

Phosphatases catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). The Commission on Enzymes of the International Union of Biochemistry has classified all these enzymes into five major groups (Florkin and Stotz, 1964). These are the phosphoric monoester hydrolases (EC 3.1.3), phosphoric diester hydrolases (EC 3.1.4), phosphoric triester hydrolases (EC 3.1.5), enzymes acting on acid anhydride bonds (EC 3.6.1), and enzymes acting on P-N bonds (EC 3.9) such as phosphoamidases. Activity represented by every class has been detected in soils except for the phosphoamidases (Skujins, 1976). Much work has been done on the other phosphatases, especially the phosphoric monoester hydrolases and several reviews have been written concerning these and the other phosphatases in soil (Cosgrove, 1967; Skujins, 1967, 1976; Ramirez-Martinez, 1968; Halstead and McKercher, 1976; Kiss et al., 1975; Spier and Ross, 1978).

A considerable number of enzymes have been found to catalyze the degradation of polyphosphates by microorganisms. Polyphosphate kinase is distributed widely among microorganisms (Harold, 1965; Hoffman-Ostenhof and Slechta, 1957; Hughes et al., 1963) and catalyzes the reaction by transferring the terminal phosphoryl group from inorganic polyphosphate to an ADP forming ATP. Polyphosphate-adenosine monophosphate

phosphotransferase transfers Pi from metaphosphate to adenosine monophosphate (Winder and Denney, 1957), whereas polyphosphate glucokinase catalyzes the phosphorylation of glucose (Szymona, 1962) and polyphosphate fructokinase catalyzes the phosphorylation of fructose by transferring Pi from a polyphosphate (Grahams salt) to form fructose-6-phosphate (Szymona and Ostrowski, 1964). Another group of enzymes known as polyphosphatases catalyzes the hydrolysis of polyphosphates. Harold (1966), in his review, reported evidence that distinct polyphatases hydrolyze specific polyphosphates of varying molecular weights or structure.

Several studies have shown that polyphosphates are hydrolyzed in soils. Most of these studies, however, have been conducted on pyrophosphate and, to a lesser extent, on tripolyphosphate and trimetaphosphate. Hydrolysis of pyrophosphate in aerobic soil systems varies with soils and requires from 4 to 100 days for 50% of the pyrophosphate to be hydrolyzed (Blanchar and Hossner, 1969; Sutton et al., 1966).

At a given temperature, the overall rate of hydrolysis of polyphosphates is the result of the complex interaction of many soil factors. Early work by Sutton and Larsen (1964) provided evidence for the important role of phosphatase. They found that, as soil pH and CO_2 evolution increased, the hydrolysis rate of pyrophosphate increased. Blanchar and Hossner (1969) found the optimal pH for pyrophosphate hydrolysis to be

near neutrality in 32 midwest soils. However, liming soils caused decreased hydrolysis rates of pyrophosphate (Gilliam and Sample, 1968; Hossner and Melton, 1970). Tabatabai and Dick (1979) found significant simple correlations of pH (negative), organic C (positive), and $CaCO_3$ content (negative) with pyrophosphatase activity in a 5-h enzyme assay in the presence of buffer (pH 8).

Polyphosphates may also undergo sorption reactions with soil constituents or form complexes with cations, which may affect the concentration of polyphosphates in soil and alter the rates of hydrolysis. Sutton and Larsen (1964) reported a lower bonding energy for sorption by soils of pyrophosphate than of Pi. Hashimoto et al. (1969) reported higher adsorption of pyrophosphate than Pi by soils. Blanchar and Hossner (1969) working with an Elliot soil (silt loam, pH 5.6) found that trimetaphosphate (TMP) was not adsorbed, but Pi, tripolyphosphate, and pyrophosphate were strongly sorbed by soils. Busman (1984) found similar results in that TMP was not sorbed by soils, whereas linear polyphosphates and Pi were sorbed by soils in the following order: $P_2 > P_3 > P_{15} \geq Pi > P_{45} > P_{65}$. Philen and Lehr (1967) showed that NH_4-pyrophosphate and NH_4-polyphosphate react much slower with clay minerals (gibbsite, goethite, montmorillonite, and attapulgite) and hydrous oxides of Fe and Al than Pi, which reacted almost immediately. Hashimoto et al. (1969) found that the reaction product in soils of

NH_4-pyrophosphates to be $Al(NH_4)_2P_2O_7OH \cdot 2H_2O$. In calcareous soils, pyrophosphate and tripolyphosphate have been shown to react with Ca and Mg (Lindsay et al., 1962; Philen and Lehr, 1967).

According to Van Wazer (1958), temperature is the most important environmental factor influencing hydrolysis rates of polyphosphates. Sutton et al. (1966) found a soil temperature of 30-35°C to be optimum for pyrophosphate hydrolysis. Other studies have shown that optimum pyrophosphatase activity in soils occur at 50-55°C (Roche, 1950; Chang and Racz, 1977; Dick and Tabatabai, 1978). Cool temperatures have been shown to decrease the rate of hydrolysis of tripolyphosphate and pyrophosphate (Engelstad and Allen, 1971; Sutton et al., 1966).

Another important parameter that affects hydrolysis rates of polyphosphates is the oxygen content of soils. When soils are flooded, immediate changes in physical, microbiological, and chemical processes occur. Waterlogging cuts off the supply of oxygen and the dominant activity of aerobic organisms is replaced by the activity of facultative and obligate anaerobes as the primary biological population in the decomposition of organic matter (Takai et al., 1956). These organisms cause reduction of the soil environment by using the oxidized soil components as electron acceptors in respiration. Flooding an acid soil causes an increase in soil pH, and, in general, flooding soils results in an increase in soluble Mn, Fe, P,

Si, and other ionic species, and an accumulation of products of anaerobic metabolism (Ponnamperuma, 1965; Patrick and Mahapatra, 1968). The increase in water-soluble P in acid soils that are submerged is due to (1) hydrolysis of Fe (III) and Al phosphates, (2) release of Pi held by anion exchange on clay and hydrous oxides of Al and Fe (III) and (3) reduction of Fe (III) to Fe (II) with liberation of sorbed and chemically bound Pi. In alkaline soils, the pH decreases upon submergence, which increases the solubility of hydroxyapatite, and thus increases water-soluble Pi (Ponnamperuma, 1972).

Hossner and Phillips (1971) compared the hydrolysis rates of pyrophosphate in four soils under aerobic and flooded conditions. They found that, with incubations of up to 10 days, the rates of hydrolysis were faster under flooded conditions than under aerobic conditions. Half-life values for applied pyrophosphate varied from 0.6 to 3.9 days.

Another important process that determines the rates of polyphosphate hydrolysis is the chemical catalysis of polyphosphates. Van Wazer (1958) indicated that colloidal gels, complexing cations, and the ionic environment can cause significant increases in the chemical degradation of polyphosphates. Given the complexity of chemical and physical properties of soils, chemical hydrolysis of polyphosphates should be important in soils. Gilliam and Sample (1968) reported

that 25 and 50% of the total hydrolysis of pyrophosphate in Norfolk and Piedmont soils, respectively, could be attributed to chemical hydrolysis. This was determined by comparing rates of pyrophosphate hydrolysis in autoclaved and nonautoclaved soils incubated 129 days at 25°C. In a similar study, Hashimoto et al. (1969) found that 32% of the total amount of pyrophosphate hydrolyzed in 22 days in an Edina soil was attributed to chemical hydrolysis at 25°C. Busman and Tabatabai (1985) reported that an average of 60% of the total amount of TMP hydrolyzed in 28 Iowa surface soils was due to chemical hydrolysis at 37°C. In this same study, the addition of Ca^{2+} and Mg^{2+} in uniform increments resulted in a linearly increase in chemical hydrolysis of TMP.

Polyphosphates as Fertilizers

Calcium metaphosphate (CMP) was produced 40 years ago but later was discarded due to high energy costs of production and low solubility. Potassium polyphosphate (a good source of P and K) also was produced and discarded due to high energy requirements and problems of byproduct (HCl) disposal (Terman, 1975). During the 1960s, ammonium phosphate and ammonium polyphosphate (APP) fertilizers were developed from addition of NH_3 to wet-process phosphoric acid or electric furnace superphosphoric acid, respectively. In 1971, the pipe reactor was developed by TVA which allowed the manufacture of ammonium

polyphosphates from wet-process phosphoric acid having low polyphosphate content. With this development, the liquid fertilizer industry based on wet-process acid greatly increased (Young and Davis, 1980).

Various polyphosphate compounds may exist in APP and other polyphosphates that have been suggested as fertilizers. Hashimoto and Lehr (1973) reported that a typical APP fertilizer contains 41% orthophosphate, 54% pyrophosphate, 4% tripolyphosphate, and 1% tetraphosphate or longer condensed phosphates. Considerable fluctuations in these values are expected as the process of manufacturing APP fertilizer is not a precise procedure. Advantages of APP are: (1) the high P content (reduces transportation costs), (2) the water solubility (allows for use in irrigation systems), and (3) ability to carry micronutrients (Engelstad and Terman, 1980).

Some early work in greenhouse and field studies was conducted on CMP. The CMP compound was a mixture of undefined cyclic and chain polyphosphates. Results from a number of studies indicated that it is less available to plants on alkaline soils than concentrated superphosphate (CSP), and nearly equal to CSP in neutral and acid soils (Tisdale and Winters, 1953; Terman and Seatz, 1956; Allamaras and Black, 1962).

Production of APP stimulated a number of field studies on this fertilizer. Engelstad and Terman (1980), in their review, found equal availability of P from APP and CSP to crops grown

on most soils. Some favorable results with APP on neutral and alkaline soils were attributed to a response to Fe or Zn, which are found in APP.

The availability of P to plants from specific polyphosphates of known structure has largely been tested on pyrophosphate. In a greenhouse study, Hughes and Hashimoto (1971) found that soil minerals decreased the uptake of pyrophosphate by oats in the following order: no mineral > kaolinite > montmorillonite > gibbsite. A study by Blanchar and Hossner (1969) indicated that uptake of pyrophosphate by corn was slightly higher than orthophosphate (greenhouse and field). Other studies indicated no real difference between uptake of pyrophosphate and Pi by plants (Blanchar and Hossner, 1969; Engelstad and Allen, 1971; Sutton et al., 1966). In general, cool temperatures inhibit uptake of pyrophosphate (Sutton and Larsen, 1964; Engelstad and Allen, 1971; Hashimoto and Wakefield, 1974). Dash et al. (1979) compared the uptake of pyrophosphate to Pi from several flooded Indian soils by transplanted rice. They found that pyrophosphate applied to flooded soils showed lower available P immediately after transplanting than Pi, but both were equally effective in yield and P uptake. Incubating moist, acid soils amended with pyrophosphate or Pi two weeks prior to flooding increased the plant-available P and grain yield. Tests with alkaline soils showed similar results but only with pyrophosphate. In a pot study in Japan,

Tsuge and Yoshida (1958) found that under flooded conditions, sodium tripolyphosphate and sodium hexametaphosphate produced more rice grain than superphosphate and had a higher residual effect on the subsequent barley crop than superphosphate. They concluded that, in general, the ring compounds were more favorable to both crops than linear compounds.

PART I. HYDROLYSIS OF POLYPHOSPHATES BY CORN ROOTS

INTRODUCTION

Polyphosphates are widely distributed among bacteria, blue-green algae, fungi, protozoa, and algae. Metaphosphates and linear polyphosphates with chain lengths as long as 500 have been isolated from microorganisms (Harold, 1966). Although polyphosphates are transitory in soils under natural conditions, additions of excess orthophosphate (Pi) can cause increases of polyphosphate accumulation in soils. Ghonsikar and Miller (1973) found that addition of Pi to glucose-amended soils resulted in accumulation of significant amounts of acid-labile polyphosphates (up to 22 μg polyphosphate-P/g soil) after 14 days of incubation (temperature not specified). Pepper et al. (1976) found that the amount of polyphosphate accumulation was proportional to the amount of Pi added. Another source of polyphosphates in the soil environment is from the use of high-analysis fertilizers such as ammonium polyphosphate. With the continuing quest for even higher grade fertilizers, increasing amounts of fertilizer P will be supplied by condensed polyphosphates.

Availability of polyphosphates for plants is mainly controlled by hydrolysis reactions because most of the P is taken up by plants as Pi, except for pyrophosphate which might be taken up by plants in small amounts (Gilliam, 1970; Subbarao et al., 1977). This hydrolysis reaction is catalyzed by phosphatases which are found in soils and plant tissues (Tabatabai,

1982; Dick and Tabatabai, 1984). As early as 1939, McGeorge (1939) reported that the presence of plant roots (nonsterile) greatly accelerated the rate of hydrolysis of metaphosphate. Other studies utilizing corn (Zea mays), tomato (Lycopersicum esculentum), and wheat (Triticum aestivum) and soybeans (Glycine max) have shown that organic P compounds such as glycerol phosphates and p-nitrophenyl phosphate can be hydrolyzed by roots (Rogers et al., 1940; Estermann and McLaren, 1961; Juma, 1976). Gilliam (1970) reported that 40 to 55% of the pyrophosphate in the presence of nonsterile wheat, corn, and barley (Hordeum vulgare) roots were hydrolyzed in a 24-h period. Studies on hydrolysis of β-glycerolphosphate (Estermann and McLaren, 1961), pyrophosphate and tripolyphosphate (Savant and Racz, 1972) by sterile and nonsterile soils indicated that rhizoplane microorganisms can increase the rates of hydrolysis associated with plant roots.

Although these studies indicated plant roots can hydrolyze certain P compounds, little information is available on hydrolysis of polyphosphates by plant roots; therefore, this study was undertaken to: (1) determine hydrolysis rates of seven linear oligomers (P_2, P_3, P_5, P_{15}, P_{25}, P_{35}, and P_{65}) and one cyclic polyphosphate (trimetaphosphate) by intact sterile or nonsterile corn roots, and (2) determine the effect of pH and temperature on rates of hydrolysis of these compounds by corn roots.

MATERIALS AND METHODS

Reagents

The polyphosphate compounds used (Table 1) were reagent-grade chemicals and were obtained from the Sigma Company (St. Louis, Missouri), with the exception of pyrophosphate and tripolyphosphate which were obtained from the Fisher Company (Itasca, Illinois). These compounds were analyzed for Pi by dissolving 20 mg of polyphosphate in 25 mL of water and determining the Pi as described by Dick and Tabatabai (1977b). Total P was determined by the Murphy and Riley (1962) method after acid hydrolysis of each polyphosphate with 1 N H_2SO_4 at 80°C for 2 h.

Seed Germination

Nonsterile seedlings were prepared by placing 6 corn seeds (Zea mays Var. L. B73 x Missouri 17) inside 6 sheets of seed germinating paper (Anchor Paper Co., St. Paul, Minnesota) and folding this into a bundle which was moistened, placed in a polyethylene bag and then incubated at 30°C for 7 days. Sterile seedlings were prepared as described by Dick et al. (1983).

Table 1. Polyphosphate compounds used

P compound			Total P		
Name	Formula	Abbreviation	Calculated[a]	Determined	Free PO_4^{3-}-P
			----------%	----------	
Sodium pyrophosphate	$Na_4P_2O_7 \cdot 10H_2O$	P_2	13.9	13.7	0.10
Sodium tripolyphosphate	$Na_4P_3O_{10}$	P_3	25.3	23.6	0.16
Sodium trimetaphosphate	$Na_3P_3O_9$	P_3, TMP	30.4	29.4	0.39
Sodium pentapolyphosphate	$Na_7P_5O_{16}$	P_5	27.1	26.9	0.11
Sodium 15-polyphosphate	$Na_{17}P_{15}O_{46}$	P_{15}	29.2	28.2	0.05
Sodium 25-polyphosphate	$Na_{27}P_{25}O_{76}$	P_{25}	29.7	28.9	0.02
Sodium 35-polyphosphate	$Na_{37}P_{35}O_{106}$	P_{35}	29.9	30.2	0.02
Sodium 65-polyphosphate	$Na_{67}P_{65}O_{196}$	P_{65}	30.1	29.3	0.01

[a]Calculated from the molecular formula.

Root Homogenate Studies

All procedures outlined below were conducted in a sterile chamber. A 12.5-g amount of sterile corn root (with seed excised) was placed in a sterile mortar along with 5 mL sterile water, ground to a liquid slurry with a sterile pestle, quantitatively transferred to a sterile 250-mL volumetric flask and diluted to volume with sterile water to make a 50 mg/mL root homogenate solution. This was then stored at 4°C.

A polyphosphate solution (3 m$\underline{M}$) was prepared by dissolving the appropriate amount of each compound in 10 mL of Modified Universal Buffer (MUB) stock solution (Skujins et al., 1962) in a 50-mL beaker. This solution was titrated to the desired pH, quantitatively transferred into a 50-mL volumetric flask and taken to volume with sterile water.

The assay for polyphosphate hydrolysis was conducted by adding 1 mL polyphosphate solution (3 m$\underline{M}$), 1 mL root homogenate, and 1 mL MUB solution to a 50-mL plastic centrifuge tube (the concentration of polyphosphate in this mixture was 1 m$\underline{M}$). The tube was swirled and immediately incubated for 1 h at 37°C with the exception of the temperature study where the incubation temperature was varied. After incubation, 25 mL cold (4°C) 1 $\underline{N}$ H_2SO_4 and 1 mL sterile water was added to each tube, the contents were mixed and filtered. An aliquot (2 mL) was immediately removed and analyzed for Pi as described by Dick and Tabatabai (1977b).

For control, the above procedure was used but 1 mL of sterilized water was added during the incubation instead of the polyphosphate solution and 1 mL of polyphosphate solution was added just before the addition of 25 mL of 1 N H_2SO_4. All experiments were run in duplicate.

Intact root studies

Polyphosphate solutions were prepared to make a 50-mg P/L solution by dissolving each compound in 200 mL MUB stock solution, tritrating to the desired pH, quantitatively transferring to a 1-L flask and diluting to volume. These solutions were cold sterilized by filtering through a sterile 0.2-mm Metrical GA-8 membrane filter (Gelman Sci. Inc., Ann Arbor, Michigan) into a sterile suction flask. An aliquot (460 mL) of the solution was transferred into a 16-oz (ca. 476 mL) sterile French square bottle containing a sterile magnetic stirrer. Each sterile corn seedling was excised at the lower end of the coleoptile, then carefully wrapped in sterile cotton and inserted into 1 of the 2 holes in a sterile No. 8 rubber stopper. The suspended root was carefully lowered into the French square bottle containing the polyphosphate solution. Both holes were sealed with a sterile rubber plug. The bottles were incubated at 30°C for 48 h. The solution was sampled at 6- and 8-h intervals by placing the bottle on the magnetic stirrer and stirring the solution slowly. Then, a sterile disposable pipet was inserted through the second hole in the

stopper and an aliquot ranging from 0.5 to 5 mL was removed and analyzed for Pi as described by Dick and Tabatabai (1977b). Controls for chemical hydrolysis were included by placing 10 mL of each polyphosphate solution in a sterile 50-mL flask which was then stopped and incubated along with the intact root bottles. Controls were also analyzed for Pi at 6- or 8-h intervals. After completion of the experiment, the roots were excised from the seed, blotted dry, and weighed to allow for calculation of the amount of Pi released in mol Pi/g corn root. This calculation also accounted for volume changes and corrected for chemical hydrolysis. All intact root experiments were run in duplicate.

In both root homogenate studies (except for the optimal pH study) and intact root studies, the buffer pH for P_3, P_5, and TMP was 5.0, whereas for P_2, P_{15}, P_{25}, P_{35}, and P_{65}, it was 6.0.

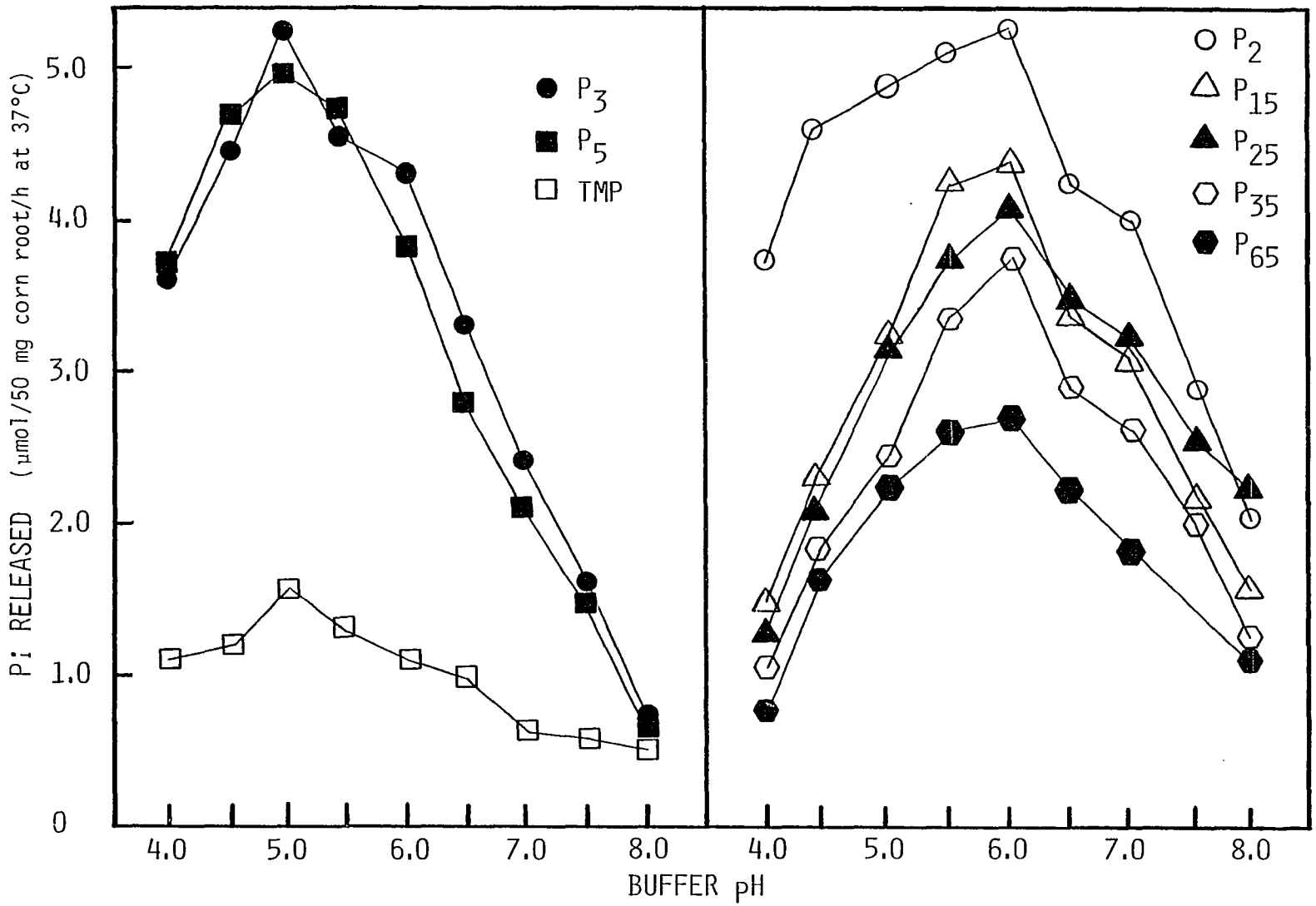
RESULTS AND DISCUSSION

Optimum pH

The buffer pH is an important parameter in studies of enzyme-mediated reactions. The hydrogen ion concentration influences the ionizable groups of the enzyme and its substrate. Both of these must be in their proper ionization state in order to maintain the conformation of the enzyme active sites and to allow for binding of the substrate. In this study, the hydrolysis of polyphosphates by corn-root homogenate was studied at buffer pH values ranging from 4 to 8 at 0.5 increments. Figure 1 shows that, for the polyphosphates P_3, P_5, and TMP, the optimal pH was 5.0, whereas for P_2, P_{15}, P_{25}, P_{35}, and P_{65}, the optimal pH was 6.0. These results correspond to those of other root studies on hydrolysis of organic P compounds where optimal pH values were found to be <7.0, indicating that acid phosphatase is catalyzing this reaction (Estermann and McLaren, 1961; Hall and Butt, 1968; Reid and Bieleski, 1970). However, Bieleski (1974) found that alkaline phosphatase activity can be induced in P-deficient Spirodela oligorrhiza (duckweed).

The shape of the pH curve varied among polyphosphates (Fig. 1). A comparison of hydrolysis rates at pH <5.0 showed that the low molecular weight compounds (P_2, P_3, and P_5) had substantially higher rates of hydrolysis than the high molecu-

Figure 1. Effect of pH of buffer on the rate of hydrolysis of polyphosphates by corn-root homogenate


Pi RELEASED (µmol/50 mg corn root/h at 37°C)
5.0
4.0
3.0
2.0
1.0
0
P3
P5
TMP
P2
P15
P25
P35
P65
4.0
5.0
6.0
7.0
8.0
BUFFER pH

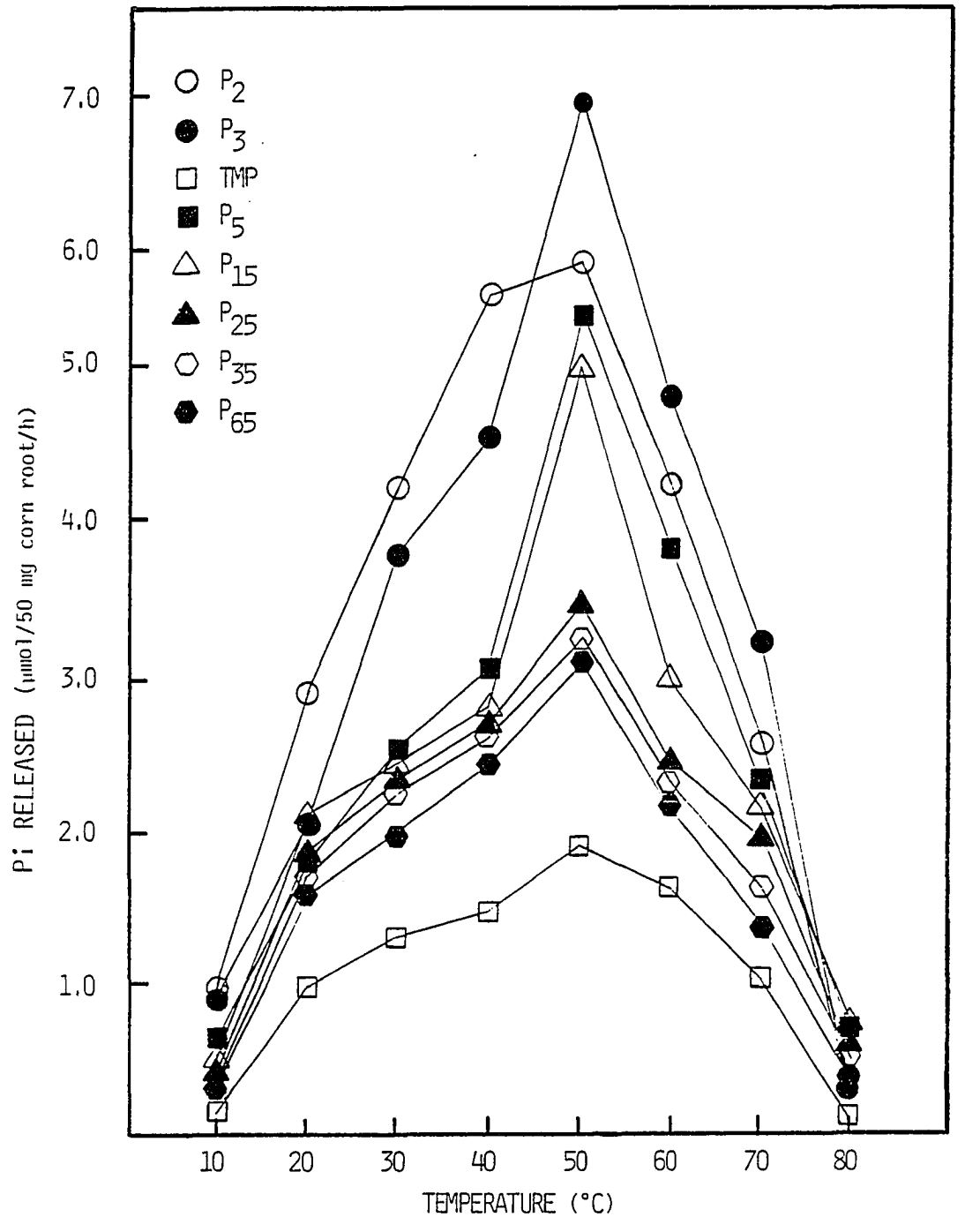
lar weight compounds (P_{15}, P_{25}, P_{35}, and P_{65}). The fact that the optimal pH of hydrolysis of the P compounds varied and that, at low pH values (e.g., pH 4), the hydrolysis rates of P_2, P_3, and P_5 were markedly different from those of P <15 indicates that there may be different phosphatases acting on different polyphosphates or that the catalytic efficiency of the phosphatases were affected by the substrate chain length.

Effect of Temperature

Enzyme-mediated reactions are temperature dependent and typically show a bell-shaped curve. Figure 2 shows such a curve for hydrolysis of polyphosphates in root homogenate. Inactivation of the enzymes responsible for polyphosphate hydrolysis occurs above the optimal temperature of 50°C. This optimal temperature is substantially higher than that reported by Estermann and McLaren (1961) who found that intact sterile barley roots had optimal β-glycerolphosphatase activity at 38°C.

Temperature can also affect chemical hydrolysis rates. Consequently, controls were run in these experiments to account for any chemical hydrolysis of the polyphosphates. For the controls, chemical hydrolysis was insignificant at temperatures of 50°C but increased as temperature of incubation increased. Since chemical hydrolysis of polyphosphates was reproducible, enzyme-mediated reaction rates could be calcu-

Figure 2. Effect of temperature of incubation on the rate of hydrolysis of polyphosphates by corn-root homogenate


P2
P3
TMP
P5
P15
P25
P35
P65
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Pi RELEASED (µmol/50 mg corn root/h)
10
20
30
40
50
60
70
80
TEMPERATURE (°C)

lated after correcting for chemical hydrolysis.

The temperature dependence of enzymatic reactions upon rate constants at temperatures below the inactivation temperature can be described by the Arrhenius equation:

$$k = A \exp(-Ea/RT)$$

where A is the pre-exponential factor, Ea is the energy of activation, R is the gas constant, and T is the temperature in °K. The Arrhenius equation can also be expressed in the log form:

$$\log k = (-Ea/2.303\ RT) + \log A$$

where log A and Ea can be determined from the intercept and slope, respectively, of a linear plot of log k vs 1/T. The activation energies in Table 2 were calculated from an Arrhenius plot utilizing the log k values at the corresponding temperatures of 10, 20, 30, 40, and 50°C.

The Ea indicates the energy barrier that must be overcome before a reaction can proceed. The Ea can be greatly reduced in the presence of a catalyst such as an enzyme. This is shown by the results in Table 2 where the Ea ranged from 32.4 to 52.5 kJ/mol for the polyphosphates tested, which are substantially lower than the chemical hydrolysis of pyrophosphate in sterile water (121 kJ/mol) reported by Weil-Malherbe and Green (1951). The Ea values of the linear polyphosphates were quite similar, ranging from 32.4 (P_2) to 40.2 (P_{15}) kJ/mol. However, the Ea value for the cyclic compound TMP was consid-

Table 2. Energy of activation and Q_{10} values of phosphatase from corn-root homogenate on polyphosphate substrates

Compound	Activation energy (kJ/mol)	Q_{10}[a] temperature (°C) indicated			
		20	30	40	50
P_2	32.4	3.03	1.46	1.30	1.05
P_3	37.1	2.20	1.88	1.19	1.52
TMP	52.5	10.88	1.34	1.14	1.46
P_5	36.9	2.89	1.37	1.20	1.92
P_{15}	40.2	3.18	1.55	1.14	1.92
P_{25}	38.0	3.46	1.66	1.18	1.25
P_{35}	37.7	4.25	1.76	1.19	1.19
P_{65}	37.8	3.25	1.72	1.24	1.29

[a] $Q_{10} = \frac{\text{hydrolysis rate } °T}{\text{hydrolysis rate } °T\text{-}10°C}$.

erably higher (52.5 kJ/mol). This reflects the additional energy that is required to open the ring structure of TMP before cleavage of the orthophosphate groups can proceed. These results are similar to those reported for pyrophosphatase activity in corn tissue (35.7 kJ/mol) and that of acid phosphatase activity in corn-root homogenate (50.1 to 51.5 kJ/mol) (Dick and Tabatabai, 1984; Juma, 1976).

The temperature coefficient (Q_{10}) is an indicator of whether a reaction is chemically or enzymatically mediated

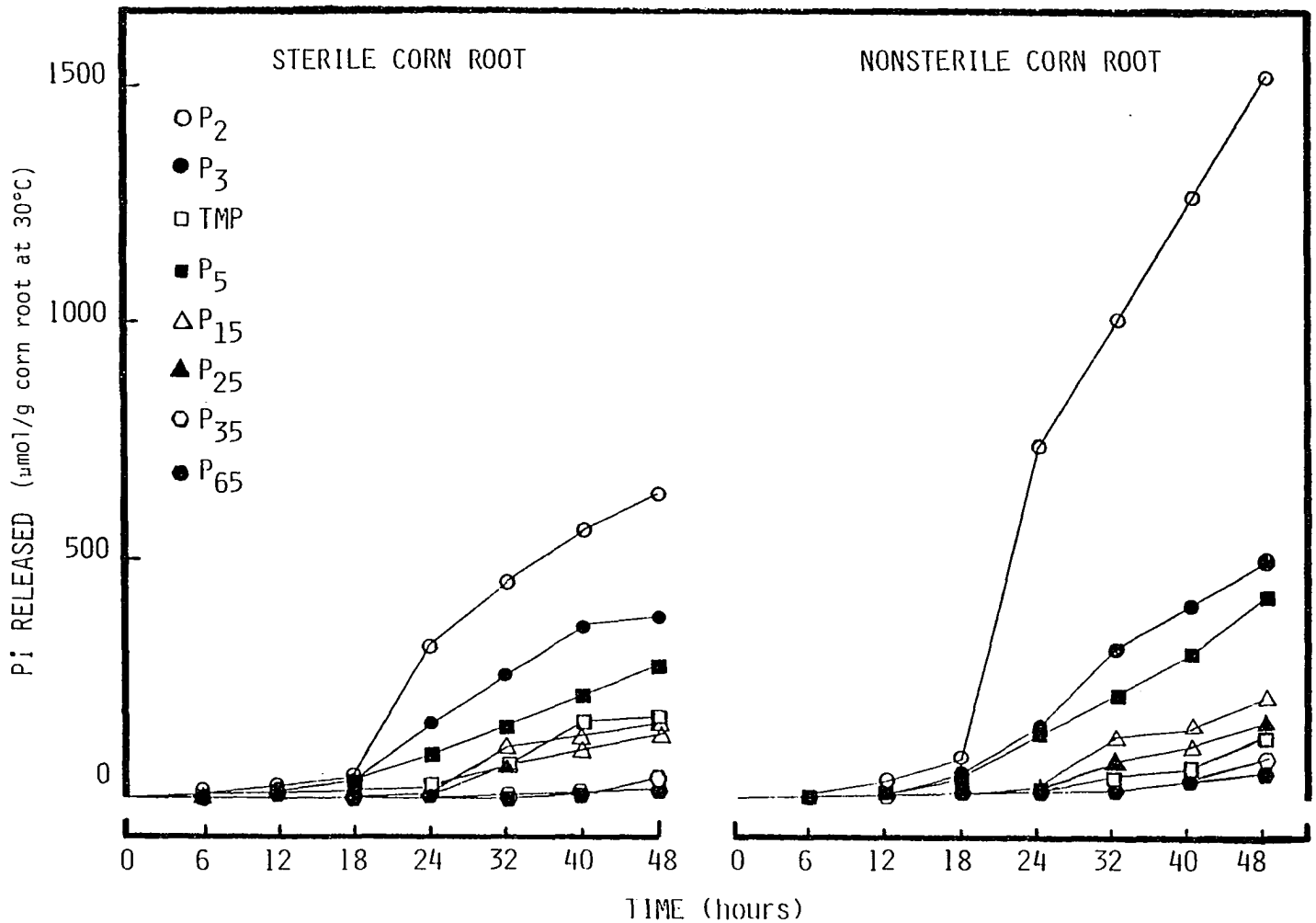
and also reflects the amount of kinetic energy required for a reaction to proceed. Normally, chemical reactions have Q_{10} values ≥ 2, whereas enzyme-catalyzed reactions, being less sensitive to temperature changes, have Q_{10} values <2. The Q_{10} values (Table 2) from 30 to 50°C were <2, indicating that the hydrolysis reaction of polyphosphates is largely enzyme-mediated and requires low kinetic energy for catalysis to occur. However, at 20°C, the Q_{10} values were >2, indicating that chemical hydrolysis was dominant and that root phosphatase activity was less important at temperatures between 10 and 20°C.

Sterile and Nonsterile Root Hydrolysis

The hydrolysis of polyphosphates by intact roots was conducted by suspending 7-day-old sterile or nonsterile corn roots in polyphosphate solutions (50 g polyphosphate-P/mL) incubated at 30°C for up to 48 h. A preliminary experiment was conducted to determine the effect of aeration on rates of hydrolysis. Results showed no differences in terms of rates of hydrolysis between aerated and unaerated roots, and visually both sets of roots appeared healthy for the duration of the 48-h period. Consequently, subsequent studies were conducted without aeration, which reduced the chances for microbial contamination.

Figure 3 shows that sterile corn roots hydrolyzed polyphosphates, indicating the presence of phosphatase activity at

Figure 3. Effect of time of incubation on the rate of hydrolysis of polyphosphates by sterile and nonsterile corn roots


STERILE CORN ROOT
NONSTERILE CORN ROOT
P_2
P_3
TMP
P_5
P_{15}
P_{25}
P_{35}
P_{65}
Pi RELEASED (µmol/g corn root at 30°C)
1500
1000
500
0
0 6 12 18 24 32 40 48
TIME (hours)

the root surface. Rates of hydrolysis decreased with increasing chain length of the polyphosphates under sterile and nonsterile conditions, with P_{35} and P_{65} being quite resistant to hydrolysis. Trimetaphosphate consistently showed lower rates of hydrolysis than its linear counterpart, P_3. This again indicates that an additional step is required to open the ring structure of TMP, thus reducing the hydrolysis rate in comparison with P_3. These results are consistent with those of Savant and Racz (1972) who found that roots of sterile wheat and pea (Pisum sativum) seedlings could hydrolyze pyrophosphate and tripolyphosphate.

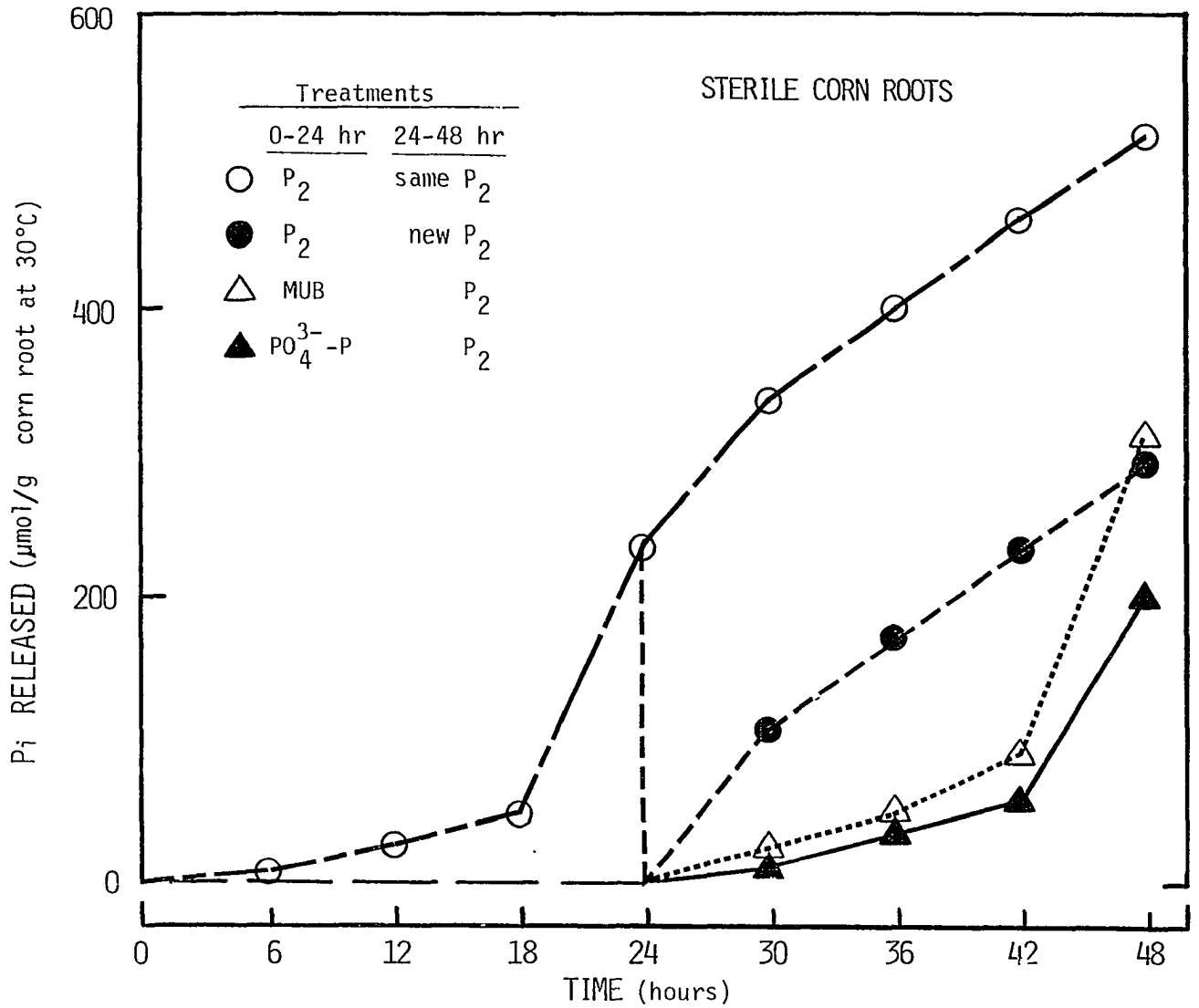
The rates of hydrolysis were higher under nonsterile than sterile conditions (Fig. 3) for all linear polyphosphates. This was particularly evident for P_2 which released greater than twofold more Pi after 48 h of incubation under nonsterile conditions than under sterile conditions. Thus, rhizoplane organisms contribute enzymatic activity. This is consistent with other studies on β-glycerolphosphate, pyrophosphate, tripolyphosphates and organic P compounds (Estermann and McLaren, 1961; Savant and Racz, 1972; McGeorge, 1939). However, TMP, the cyclic compound, had higher rates of hydrolysis under sterile conditions. It is difficult to explain why TMP was an exception, but the results may suggest that there is some type of interaction between TMP and rhizoplane organisms that caused a decrease in rates of hydrolysis.

Effect of Substrates on Induction of Pyrophosphatase

The rates of hydrolysis varied over time (Fig. 3). For the first 18 h, the rate of hydrolysis was extremely slow, but after 18 h, the rate of hydrolysis increased significantly for the P compounds of chain length $\leq P_{25}$. This suggested that the phosphatases associated with polyphosphate hydrolysis were induced in the presence of their substrates. Consequently, an experiment was conducted to confirm this conclusion.

In this experiment, sterile intact corn roots were suspended in a pyrophosphate solution (50 mg P/L) and the amount of Pi produced in 6-h intervals was determined up to 48 h. The results (open circles) showed a lag period of 18 h after which the hydrolysis rate increased (Fig. 4). In a second set of duplicated bottles, sterile corn roots were suspended in a pyrophosphate solution for 24 h, the roots were removed, washed and resuspended in a fresh pyrophosphate solution. The amount of Pi produced from these roots in the fresh pyrophosphate solution did not show a lag period (solid circles) during the second 24-h incubation period, indicating that pyrophosphatase was induced during the initial 18 h in the presence of its substrate. Further tests showed that the MUB had no effect on the initial rate of pyrophosphate hydrolysis because addition of the substrate after suspending the roots in MUB again showed that 18 h were required for induction of pyrophosphatase

Figure 4. Effect of substrate on the induction of pyrophosphatase by intact sterile corn roots: ○ , roots in pyrophosphate solution (50 mg P/L) for 48 h; ● , the pyrophosphate solution was changed after 24 h; △, roots were placed in pyrophosphate solution after 24 h in MUB buffer; ▲ , roots were placed in pyrophosphate solution after 24 h in orthophosphate solution (50 mg P/L)


STERILE CORN ROOTS
Treatments
0-24 hr
24-48 hr
P2
same P2
P2
new P2
MUB
P2
PO4 3- -P
P2
Pi RELEASED (µmol/g corn root at 30°C)
600
400
200
0
0
6
12
18
24
30
36
42
48
TIME (hours)

in corn roots (open triangles). Results showed that, when roots were suspended in Pi (50 mg P/L) solution (solid triangles) during the first 24-h period and then immersed in the pyrophosphate solution, an 18-h lag period again occurred. Figure 4 shows that Pi did not affect the lag period but decreased pyrophosphatase activity. This is not surprising because it is well known that Pi is a competitive inhibitor of pyrophosphatase.

PART II. HYDROLYSIS OF POLYPHOSPHATES IN SOILS

INTRODUCTION

Condensed inorganic phosphates (polyphosphates) are generally characterized as pentavalent phosphorus (P) compounds in which various numbers of tetrahedral orthophosphate (Pi) groups are linked by oxygen bridges within a linear or cyclic configuration (Thilo, 1962). These linear oligomers and cyclic polyphosphates occur in all biological systems (Harold, 1966) but are transitory in soils (Ghonsikar and Miller, 1973). Polyphosphates also can accumulate in soils with the addition of excess Pi. Pepper et al. (1976) found that additions of from 100 to 1000 μg Pi/g soil resulted in accumulation of acid-labile polyphosphate (6.3 to 53.8 μg P/g soil).

Polyphosphates are of interest as P fertilizer sources because of their water solubility and high P content. Numerous greenhouse and field studies have shown that polyphosphates provide adequate amounts of Pi to plants and compare favorably with conventional P fertilizers (Englestad and Terman, 1980). Most of these studies, however, were conducted with ill-defined polyphosphates or with low-molecular-weight compounds such as pyrophosphate. Early studies with British soils showed that the hydrolysis of pyrophosphate was largely a function of pH (hydrolysis increased with increasing pH) and biological activity (hydrolysis increased as CO_2 evolution increased) and that cool temperatures retarded hydroly-

sis (Sutton and Larsen, 1964; Sutton et al., 1966). Studies on hydrolysis of pyrophosphate under aerobic conditions indicated that a fraction of the applied pyrophosphate (10 to 20%) persists in soils, presumably owing to formation of insoluble reaction products (Gilliam and Sample, 1968; Hossner and Melton, 1970).

The availability of Pi for plant uptake is largely limited by sorption mechanisms in soils. Among the polyphosphates, trimetaphosphate (TMP) has a unique characteristic of not being sorbed by soil constituents (Blanchar and Hossner, 1969; Busman, 1984). However, TMP in soils is hydrolyzed biochemically or chemically to tripolyphosphate, pyrophosphate, and ultimately, to Pi, all of which are susceptible to fixation by soil constituents (Blanchar and Hossner, 1969; Busman and Tabatabai, 1985).

The fate of polyphosphates in soils is of interest because sorption reactions and hydrolysis of these compounds would affect the P availability for plants. Because limited information is available on hydrolysis of various polyphosphates in soils, this study was conducted with the following objectives in mind: (1) to compare the rates of hydrolysis of seven linear oligomers (P_2, P_3, P_5, P_{15}, P_{25}, P_{35}, P_{65}) and of one cyclic polyphosphate (TMP) in soils and (2) to study the kinetic properties of hydrolysis of these compounds under aerobic and waterlogged conditions.

MATERIALS AND METHODS

Surface soils (0-15 cm) were selected to give a range in pH, organic matter content, organic P, inorganic P, texture, and $CaCO_3$ content (Table 3). Field-moist soil samples were collected and immediately sieved to pass a 5-mm screen and divided into two portions. One portion was placed in a polyethylene bag and stored at 4°C. The second portion was spread on clean paper and air-dried at room temperature (22°C). A part of the air-dried sample was ground to pass an 80-mesh sieve, and the rest was crushed to pass a 2-mm screen.

The polyphosphates used (Table 4) were reagent grade. With the exception of pyrophosphate and tripolyphosphate, which were obtained from the Fisher Scientific Co. (Itasca, Illinois), the polyphosphates were obtained from Sigma Chemical Co. (St. Louis, Missouri). The percentage of total P and free Pi in each compound used are reported in Table 1, Part I.

For the soil properties reported in Table 3, pH was determined by a combination glass electrode (soil:water ratio, 1:2.5), organic C by the method of Mebius (1960), total P by the method of Dick and Tabatabai (1977a), and inorganic P as described by Olsen and Dean (1965). Organic P was determined by subtracting the value of inorganic P from that obtained for total P. Inorganic C was determined by the method of Bundy and Bremner (1972). The cation-exchange capacity was measured by the method of Chapman (1965). Exchangeable K, Ca, and Mg

Table 3. Properties of soils used

Soil	Subgroup	pH	Organic C	$CaCO_3$ equiv.
			--------%-------	
Clarion	Typic Hapludolls	5.8	1.43	0
Webster	Typic Haplaquolls	6.2	3.32	0
Muscatine	Aquic Hapludolls	6.4	2.05	0
Canisteo	Typic Hapludolls	7.8	4.12	7.1

[a]mol (NH_4^+)/g soil.

[b]ND, not determined (calcareous soil).

Phosphorus		Exchangeable					
Org.	Inorg.	K	Ca	Mg	CEC[a]	Clay	Sand
-mg/kg soil-		---mol (i)/g soil---				------%-----	
233	210	2.5	110	32	163	27	62
368	114	5.0	220	56	299	30	41
322	184	10.2	206	54	284	33	10
846	344	3.3	ND[b]	ND	ND	37	8

Table 4. Polyphosphate compounds used

Name	Formula	Abbreviation
Sodium pyrophosphate	$Na_4P_2O_7 \cdot 10H_2O$	P_2
Sodium tripolyphosphate	$Na_4P_3O_{10}$	P_3
Sodium trimetaphosphate	$Na_3P_3O_9$	P_3, TMP
Sodium pentapolyphosphate	$Na_7P_5O_{16}$	P_5
Sodium 15-polyphosphate	$Na_{17}P_{15}O_{46}$	P_{15}
Sodium 25-polyphosphate	$Na_{27}P_{25}O_{76}$	P_{25}
Sodium 35-polyphosphate	$Na_{37}P_{35}O_{106}$	P_{35}
Sodium 65-polyphosphate	$Na_{67}P_{65}O_{196}$	P_{65}

were determined by using 1 N neutral NH_4OAc as described by Heald (1965) and Pratt (1965), and particle-size distribution by the pipette method of Kilmer and Alexander (1949). Organic C, total P, inorganic P, and total N were performed on the <80-mesh samples. All other analyses and experiments were performed on the <2-mm mesh soil samples.

The rates of hydrolysis of the polyphosphates in soils were determined under aerobic and waterlogged conditions. In aerobic incubations, 2 g of soil were placed in a 50-mL plastic centrifuge tube and treated with 1 mL of polyphosphate solution containing 1 mg P of a specific compound (500 μg P/g soil). This solution was added dropwise to uniformly

moisten the whole soil sample (70% of water-holding capacity). The tube was then stoppered and incubated at a specified temperature for times ranging from 1 to 14 days. The tube was aerated daily by removing the stopper and flushing the tube with air. The moisture content was maintained by weighing tubes daily and adding the required amount of water. The incubation under waterlogged conditions was performed by adding the 1 mg of the polyphosphate-P in a 5-mL solution, and the tube was not aerated. After incubation, 1 mL of water was added for the aerobic treatment, or 5 mL of water for the waterlogged treatment, and the orthophosphate (Pi) produced was extracted with 1 $\underline{N}$ H_2SO_4 and determined as described by Dick and Tabatabai (1978).

Controls were performed to correct for the native Pi in each soil and for any trace amounts of Pi that might be present in the polyphosphates. For the control, 2 g of soil were treated with 1 mL or 5 mL of water and incubated along with the polyphosphate-treated soils. After incubation, a 1-mL or 5-mL solution containing 1 mg of the corresponding polyphosphate-P was added to the control incubated under aerobic or waterlogged conditions, respectively. The Pi was extracted and determined as previously described for the polyphosphate-treated samples.

All values reported are averages of duplicate determina-

tions expressed on an oven-dry weight basis, with moisture being determined from loss in weight after drying at 105°C for 48 h.

RESULTS AND DISCUSSION

Effect of Air Drying

The effect of air drying field-moist soils on hydrolysis of the polyphosphates under aerobic conditions after incubation at 25°C for 7 days is shown in Table 5. In general, the amounts of Pi produced per kg of soil in both field-moist and air-dried soils decreased as the chain length of the polyphosphate increased. Expressed as percentage of the amount of Pi produced in field-moist soils, the amount of Pi produced in air-dried soils ranged from 66% with P_3 added to Muscatine soil to 96% with TMP added to the calcareous Canisteo soil. The information available indicates that the effect of air drying of field-moist soils on enzyme activity varies with different enzymes (Skujins, 1967). The results obtained in this study (Table 5) support the work of Tabatabai and Dick (1979), who reported that air drying decreased pyrophosphatase activity from 23 to 40% in Iowa surface soils. Because it is known that polyphosphates are hydrolyzed in soils by chemical and biochemical reactions, it is very likely that the lower rates of hydrolysis in air-dried soils compared with field-moist soils are due to inactivation of exocellular or microbial phosphatases. Also, this decrease in hydrolysis in air-dried soils could be due to the increased solubility of native inorganic P compounds, which are known to inhibit

Table 5. Effect of soil pretreatment on hydrolysis of polyphosphates in soils incubated under aerobic conditions at 25°C for 7 days

P compound	Amount of orthophosphate produced in soil specified							
	Clarion[a]		Webster		Muscatine		Canisteo	
	AD[b]	FM	AD[b]	FM	AD[b]	FM	AD[b]	FM
	mg PO_4^{3-}-P/kg soil							
P_2	213 (87)	244	254 (81)	315	206 (67)	306	243 (66)	366
P_3	223 (91)	244	238 (81)	292	190 (66)	289	301 (96)	313
P_3, TMP	197 (94)	209	264 (93)	286	171 (59)	290	294 (94)	320
P_5	198 (88)	226	233 (81)	289	223 (80)	277	275 (86)	320
P_{15}	171 (88)	194	231 (87)	265	205 (79)	258	276 (84)	329
P_{25}	161 (88)	183	195 (79)	247	180 (74)	244	275 (83)	329
P_{35}	154 (94)	164	183 (75)	244	194 (89)	219	282 (91)	311
P_{65}	152 (90)	168	185 (72)	257	189 (76)	247	225 (84)	267

[a]AD, air-dried soil; FM, field-moist soil.

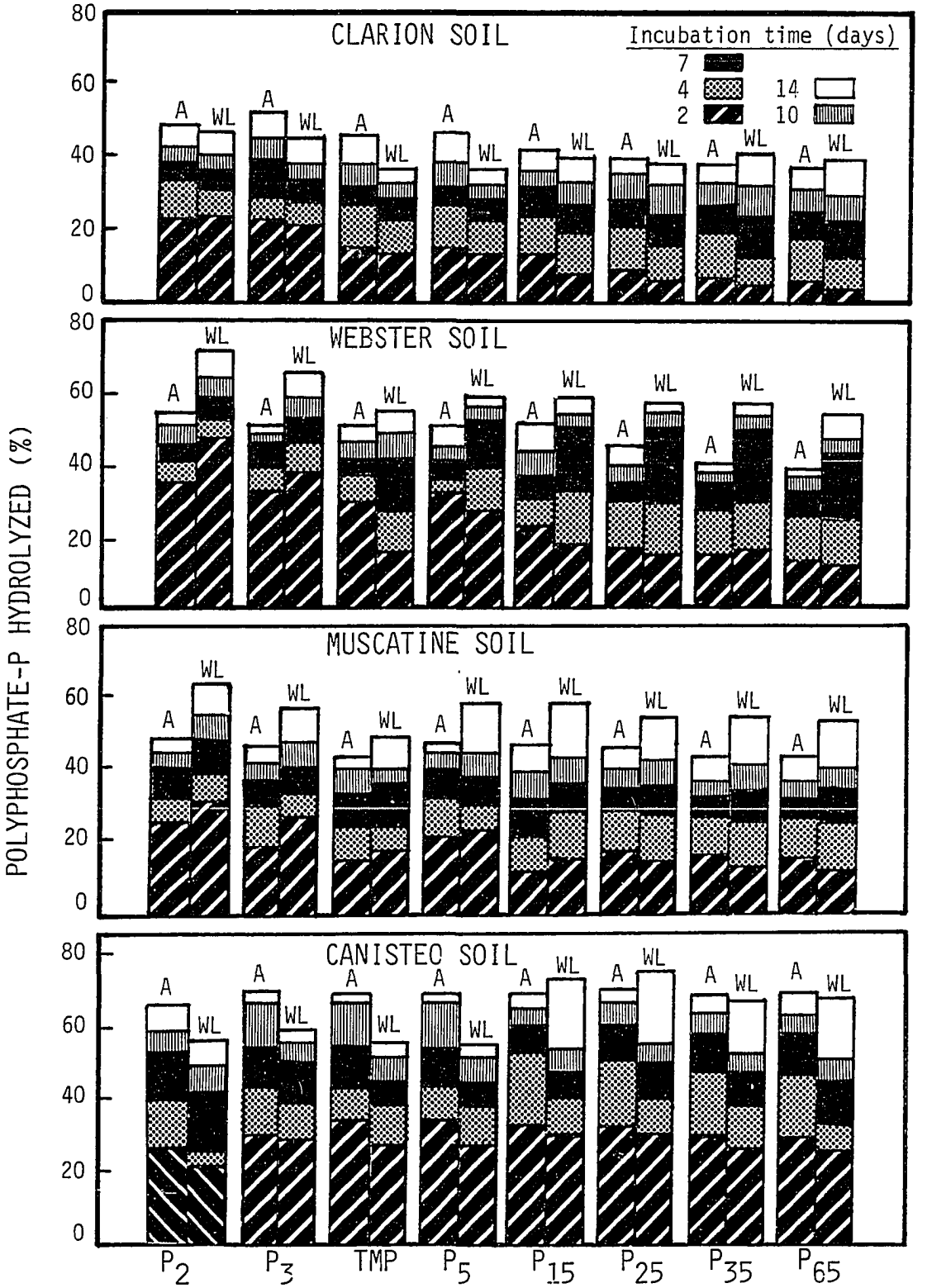
[b]Numbers in parentheses are the amounts of Pi produced in air-dried soil expressed as a percentage of that produced in field-moist soil.

phosphatase activity (Kulaev, 1979).

Effect of Incubation Time

The effect of time on hydrolysis of polyphosphates in soils incubated under aerobic and waterlogged conditions is shown in Figure 5. The percentage of the polyphosphates hydrolyzed in the four soils studied increased with time of incubation in both aerobic and waterlogged conditions and varied among the soils and the polyphosphates used. In general, the amount of Pi produced decreased with increasing polyphosphate chain length. This was especially true after 2 days of incubation in the three acid soils (Clarion, Muscatine, and Webster). No such trend in Pi production was observed in the calcareous Canisteo soil. The percentage of the polyphosphate-P hydrolyzed after 14 days of incubation did not exceed 75% of the amount added under both aerobic and waterlogged conditions (Fig. 5). There were marked variations in the amounts of polyphosphates hydrolyzed under the incubation conditions studied, but these variations were not consistent among the soils and the polyphosphates. The observed differences in the percentage of polyphosphate hydrolyzed in the four soils seemed to be related to the soil pH. Work by Dick and Tabatabai (1978) showed that optimal pyrophosphatase activity occurs at pH values between 7 and 8 in soils. A similar conclusion was reached by Sutton and Larsen (1964) on the

Figure 5. Effect of time on hydrolysis of polyphosphates in soils incubated under aerobic (A) and waterlogged (WL) conditions at 25°C


CLARION SOIL
Incubation time (days)
7
4
2
14
10
WEBSTER SOIL
MUSCATINE SOIL
CANISTEO SOIL
A
WL
POLYPHOSPHATE-P HYDROLYZED (%)
0
20
40
60
80
P2
P3
TMP
P5
P15
P25
P35
P65

hydrolysis of pyrophosphate in English soils. Conversely, hydrolysis proceeded more rapidly in acid than in alkaline Texas soils (Blanchar and Hossner, 1969). The results reported in Figure 5 show that the highest percentages of polyphosphate hydrolysis occurred in the calcareous Canisteo soil. That hydrolysis rates vary among soils, incubation conditions, and with the type of polyphosphate used, reflects the complex interaction of enzyme activity, chemical hydrolysis, and sorption reactions of the polyphosphates in soils.

The effect of flooding was very similar for the Muscatine and Webster soils (Fig. 5). These soils were intermediate in pH compared with the other two soils and showed consistently higher rates of hydrolysis with waterlogging as compared with aerobic conditions after 14 days incubation. The results with these two soils are consistent with those reported by Hossner and Phillips (1971), who found higher rates of hydrolysis of pyrophosphate under flooded conditions than those under aerobic conditions after 10 days incubation at 25°C. The variation in hydrolysis rates between aerobic and waterlogged conditions are due to changes in physical, chemical, and microbiological processes that occur in flooded soils. Changes that could affect rates of hydrolysis when a soil is flooded include a rise in pH in acid soils and solubilization of Mn and Fe (Patrick and Fontenot, 1976). Increased solubilization of metals (e.g., formation of Fe^{2+}) would decrease sorption of

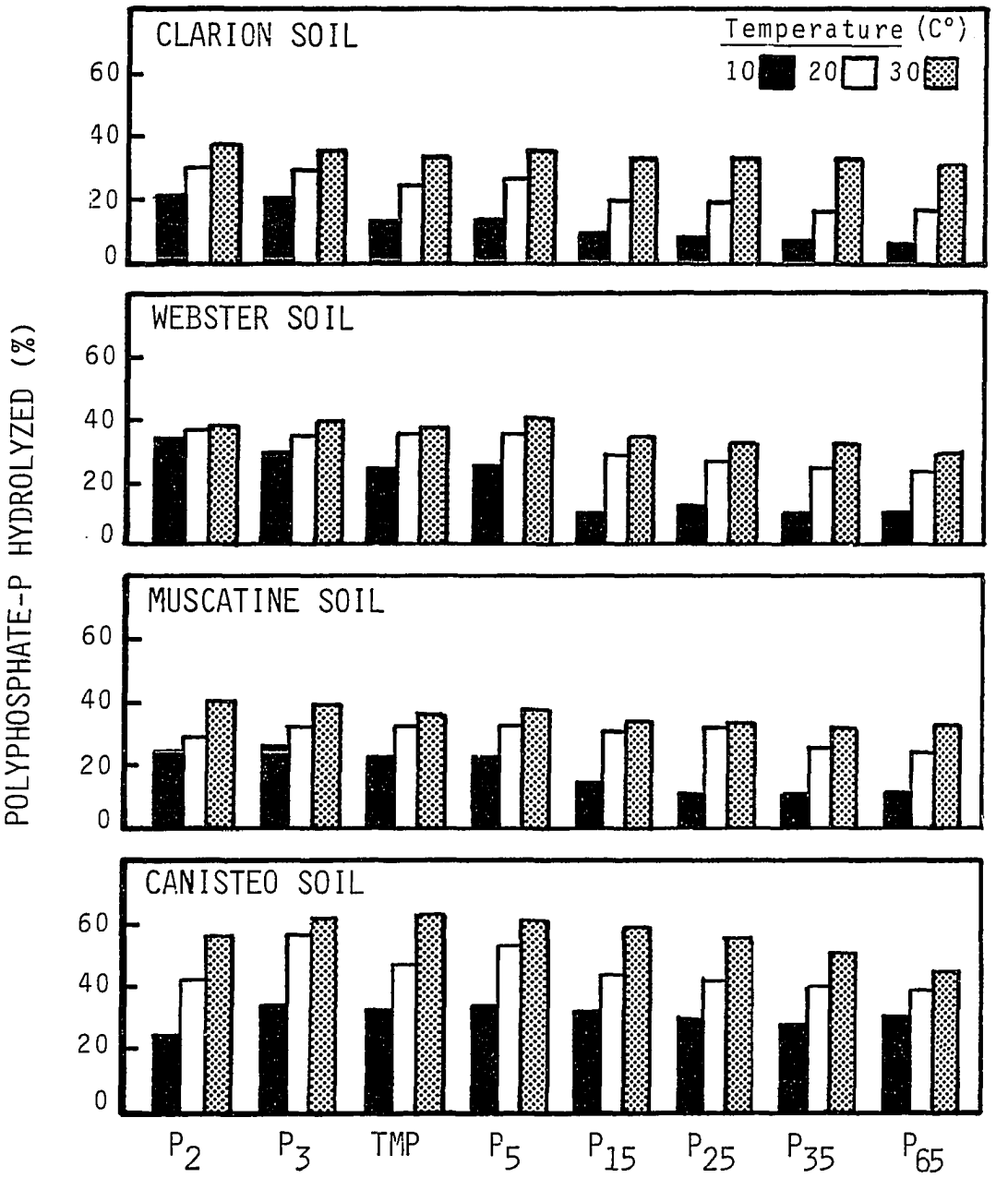
linear polyphosphates, thus leaving them susceptible for hydrolysis reactions. In contrast, the acid Clarion soil showed either nearly equal rates of hydrolysis between waterlogged and aerobic conditions (P_3, P_{15}, P_{25}, P_{35}, P_{65}) or less hydrolysis under waterlogged conditions (P_3, TMP, P_5).

During the first 10 days of incubation, the alkaline Canisteo soil showed higher rates of hydrolysis with all P compounds under aerobic conditions than under flooded conditions. After 10 days, however, there was a sharp increase in the amount of Pi released under flooded conditions with the longer linear polyphosphates (P_{15}, P_{65}), which resulted in these compounds having increased amounts of Pi released compared with lower-molecular-weight compounds.

Effect of Temperature

Temperature has a significant effect on biochemical and chemically catalyzed reactions. Figure 6 shows the effect of temperature on hydrolysis of polyphosphates in soils incubated under aerobic conditions for 7 days. Although the percentage of polyphosphates hydrolyzed varied among the four soils used, it increased with increasing temperature from 10 to 30°C for all the P compounds studied. The incubation temperatures tested (10, 20, 30°C) are those that typically occur under field conditions. Brady (1974) reported soil temperature data from Nebraska that ranged from 10 to 28°C at a depth of 7.6 cm

Figure 6. Effect of temperature on hydrolysis of polyphosphates in soils incubated for 7 days


CLARION SOIL
Temperature (C°)
10 20 30
WEBSTER SOIL
MUSCATINE SOIL
CANISTEO SOIL
POLYPHOSPHATE-P HYDROLYZED (%)
0
20
40
60
P2
P3
TMP
P5
P15
P25
P35
P65

during the growing season. This effect of temperature is significant because polyphosphates are largely taken up by plants as Pi. Gilliam (1970) using ^{32}P and Subbarao et al. (1977) using NMR spectroscopy have shown that small amounts of pyrophosphate can be absorbed by corn roots. But because of the presence of phosphatases in plant roots and soils, it is most likely that all polyphosphates are hydrolyzed to Pi before being taken up by plants. The results reported in Figure 6 show that rates of hydrolysis are substantially lower at 10°C than at 20 and 30°C, which supports the results of Sutton et al. (1966) and Hashimoto and Wakefield (1974) that cool temperatures limit the hydrolysis of pyrophosphate.

The percentage of polyphosphate hydrolyzed (Fig. 6) in the three acid soils at 10°C and 20°C decreased as the chain length of the polyphosphate increased. This was particularly evident at the incubation temperature of 10°C. For example, the percentage of the polyphosphates hydrolyzed at 10°C during 7 days in the Clarion soil ranged from 20% for P_2 to 5% for P_{65}. The effect of chain length of the polyphosphates on hydrolysis was less variable at 30°C. However, the effect of temperature on hydrolysis of the polyphosphates in the calcareous Canisteo soil was more evident at 20 and 30°C than at 10°C.

Temperature coefficient (Q_{10}) is an indicator of whether the reaction is chemically or enzymically mediated. Normally,

chemical reactions have Q_{10} values ≥ 2, whereas enzyme-catalyzed reactions, being less sensitive to temperature changes, have Q_{10} values <2 (Zeffren and Hall, 1973). From the results obtained on the effect of temperature on rate of hydrolysis of polyphosphates in soils, the Q_{10} values were calculated for 20 and 30°C (Table 6). The Q_{10} values at 30°C were <2 and uniform, indicating that the hydrolyses were largely controlled by enzyme-mediated reactions and that low kinetic energies were required for the hydrolysis reactions. Similar values were obtained for the Q_{10} values at 20°C in the Canisteo soil. The three acid soils, however, showed a distinct division in Q_{10} values at 20°C between the compounds of P_{15}, P_{25}, P_{35}, and P_{65} and those of P_2, P_3, TMP, and P_5, with Q_{10} values of the former group being >2 and the latter group <2. This difference in Q_{10} values suggests that, at temperatures between 10 and 20°C, the higher-molecular-weight polyphosphates are largely hydrolyzed by chemical catalysis in acid soils.

The energy of activation (Ea) is a measure of the energy barrier that must be overcome before the reaction can proceed. Energy of activation is derived from the temperature dependence of the rate constants of catalyzed reactions. This temperature influence can be described by the Arrhenius equation:

$$k = A \exp(-Ea/RT)$$

where k is the rate constant, A is the pre-exponential factor,

Table 6. Activation energy and the Q_{10} values for hydrolysis of polyphosphates in soils incubated under aerobic conditions

Soil	P compound	Activation energy (kJ/mol)	Q_{10} at temperature (°C) indicated[a]	
			20	30
Clarion	P_2	23.1	1.40	1.22
	P_3	24.9	1.50	1.20
	TMP	39.9	1.91	1.33
	P_5	38.1	1.81	1.35
	P_{15}	59.5	2.46	1.64
	P_{25}	71.7	3.00	1.75
	P_{35}	71.2	2.87	1.85
	P_{65}	68.8	2.81	1.79
Webster	P_2	10.7	1.19	1.05
	P_3	11.6	1.23	1.06
	TMP	14.9	1.35	1.04
	P_5	16.2	1.28	1.13
	P_{15}	51.6	2.80	1.13
	P_{25}	41.9	2.17	1.23
	P_{35}	45.7	2.47	1.18
	P_{65}	46.7	2.36	1.26
Muscatine	P_2	21.2	1.19	1.38
	P_3	18.8	1.25	1.24
	P_3, TMP	21.9	1.44	1.15
	P_5	22.4	1.47	1.15
	P_{15}	36.2	2.15	1.08
	P_{25}	51.1	2.60	1.27
	P_{35}	49.5	2.52	1.26
	P_{65}	49.4	2.40	1.33
Canisteo	P_2	29.1	1.66	1.39
	P_3	25.4	1.63	1.10
	P_3, TMP	25.4	1.52	1.18
	P_5	27.8	1.45	1.32
	P_{15}	26.7	1.40	1.33
	P_{25}	26.8	1.40	1.33
	P_{35}	25.4	1.39	1.30
	P_{65}	21.2	1.28	1.15

[a] $Q_{10} = \frac{\text{hydrolysis rate at °T}}{\text{hydrolysis rate at °T - 10°C}}$.

EA is the activation energy, R is gas constant, and T is the temperature in °K. This equation can also be expressed in the log form:

$$\log k = (-Ea/2.303RT) + \log A \quad .$$

The activation energy can be calculated from the slopes of the line when log k is plotted against 1/T. The slope of the lines obtained from plotting the log of hydrolysis rates at 10, 20, and 30°C were used to calculate the Ea (Table 6). A catalyst, such as an enzyme, greatly reduces the Ea value, thus increasing the reaction rate. For example, chemical hydrolysis of pyrophosphate is 121 kJ/mol (Weil-Malherbe and Green, 1951), whereas pyrophosphatase-catalyzed reactions in soils, plant materials, and animal manures range from 37.6 to 40 kJ/mol (Dick and Tabatabai, 1984). The Ea reported in Table 6 are substantially lower (10.7 to 71.7 kJ/mol) than the Ea reported for chemical hydrolysis of pyrophosphate (121 kJ/mol), suggesting that the polyphosphate hydrolysis is controlled primarily by enzyme-mediated reactions. In the alkaline Canisteo soil, the Ea were relatively uniform among the polyphosphates, but in the three acid soils, the Ea was substantially higher for polyphosphates with chain lengths of $\geq$15 Pi units/molecule. Thus, in the acid soils, more energy is required to initiate the hydrolysis of the longer chained polyphosphates than the lower-molecular-weight compounds. In a similar experiment, Hossner and Phillips (1971), measured

the hydrolysis of pyrophosphate in a flooded soil and reported an Ea value of 22.1 kJ/mol, which is within the range of 10.7 to 29.1 kJ/mol for P_2 shown in Table 6.

Rate Constants

Plotting the logarithm of the percentage of nonhydrolyzed polyphosphate-P against time suggests that the rate of hydrolysis of polyphosphates is controlled by two first-order reactions. This is shown in Figure 7 for the Webster soil. Similar results were obtained for the other three soils. The change in rates typically occurred between 2 and 7 days. This break usually occurred near day 2 for short-chain compounds such as P_2 and P_3, whereas the longer-chain compounds showed this change in rate between 4 and 7 days. These two first-order reactions may be the result of the greater availability of the polyphosphates for hydrolysis during the initial 1 to 4 days. But as time proceeds, the degree of adsorption and precipitation increases, which could decrease the hydrolysis of polyphosphates. Gilliam and Sample (1968) reported similar two first-order reactions for pyrophosphate hydrolysis in one of two sterile soils that they studied at 25°C.

Rate constants were calculated from the slopes of lines from plots such as Figure 7 for each soil, where k_1 is the initial faster rate and k_2 is the subsequent slower rate. Tables 7 and 8 show the rate constants for hydrolysis of

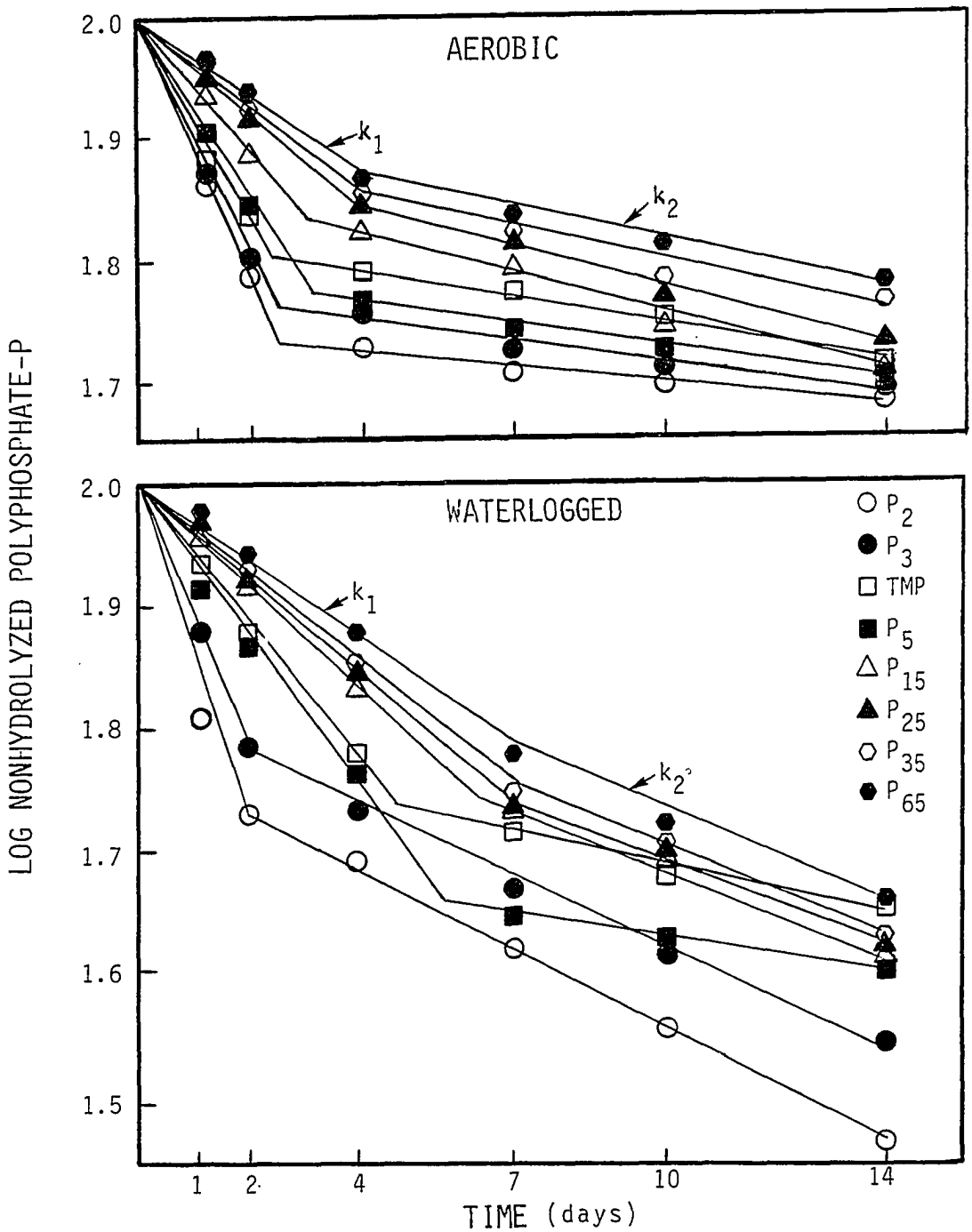


Figure 7. The relation between time of incubation and the logarithm of the percentage of polyphosphate remaining in Webster soil at 25°C

Table 7. Rate constants for hydrolysis of polyphosphates in soils incubated under aerobic conditions at 25°C

P compound	Rate constant for soil specified							
	Clarion[a]		Webster		Muscatine		Canisteo	
	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
	10^{-5}/min							
P_2	10.58	1.13	17.21	0.74	11.29	1.24	11.00	3.22
P_3	10.25	2.19	16.77	1.01	13.24	1.24	16.83	4.56
TMP	5.70	2.14	14.53	1.36	10.41	1.22	15.96	4.03
P_5	8.68	2.00	13.99	1.40	11.38	1.38	17.16	3.85
P_{15}	4.90	1.43	9.36	2.53	8.68	1.33	13.59	2.26
P_{25}	3.54	1.45	6.80	1.91	6.34	1.45	12.27	2.44
P_{35}	3.42	1.47	5.73	0.76	5.64	1.70	11.67	2.23
P_{65}	3.34	1.33	5.22	1.22	5.30	1.77	11.63	3.29

[a]For k_1 and k_2, see Figure 7.

Table 8. Rate constants for hydrolysis of polyphosphates in soils incubated under waterlogged conditions at 25°C

P compound	Rate constant for soil specified							
	Clarion[a]		Webster		Muscatine		Canisteo	
	k_1	k_2	k_1	k_2	k_1	k_2	k_1	k_2
	10^{-5}/min							
P_2	10.79	1.77	24.91	3.38	14.17	3.86	7.71	2.74
P_3	8.17	1.33	17.73	3.18	12.44	3.02	7.73	5.09
TMP	2.80	1.06	9.11	1.43	5.73	2.16	8.98	2.56
P_5	3.63	1.86	9.85	2.60	9.04	3.27	10.25	2.58
P_{15}	3.33	1.75	6.76	2.51	5.18	3.02	12.84	4.70
P_{25}	2.89	1.98	6.54	2.14	4.88	3.36	11.04	5.23
P_{35}	2.69	2.21	5.77	2.69	4.72	3.34	11.72	4.28
P_{65}	2.58	2.19	5.00	2.60	4.74	2.95	12.23	4.58

[a]For k_1 and k_2, see Figure 7.

polyphosphates under aerobic and waterlogged conditions, respectively. The rate constants under aerobic conditions ranged from 7.40×10^{-6} to 1.72×10^{-4}/min, whereas the rate constants under waterlogged conditions ranged from 1.06×10^{-5} to 2.49×10^{-4}/min. These first-order rate constants are higher than those reported for hydrolysis of pyrophosphate in distilled water (25°C, pH 7.0), with rates on the order of 4.5×10^{-7} to 6.4×10^{-7}/min (Clesceri and Lee, 1965) or those reported for autoclaved soil samples (1.0×10^{-6} to 5.9×10^{-6}/min) (Gilliam and Sample, 1968). Hossner and Phillips (1971) reported rate constants for pyrophosphate hydrolysis ranging from 1.25 c 10^{-4} to 3.72×10^{-5}/min for four flooded Texas soils at 25°C. This compares favorably with the P_2 hydrolysis shown in Table 8, where the k_1 and k_2 values ranged from 2.49×10^{-4} to 1.77×10^{-5}/min at 25°C under flooded conditions.

The hydrolysis rates under aerobic conditions at 25°C were 16-268 and 7-29 times faster than hydrolysis rates of pyrophosphate in sterile water and autoclaved soils, respectively. Corresponding rates of hydrolysis under waterlogged conditions were 24-389 and 11-42 times faster than hydrolysis of pyrophosphate in sterile water and autoclaved soils. These comparisons of rates for flooded soils at 25°C are similar to those reported by Hossner and Phillips (1971), who found that rates of hydrolysis of pyrophosphate were 70-230 times faster

than hydrolysis in sterile water and 10-40 times faster than in autoclaved soils. This indicates the important role of phosphatases in mediating hydrolysis of polyphosphates in soils.

Comparison of the k_1 and k_2 values of the hydrolysis of polyphosphates in each soil showed a general decrease in rates of hydrolysis with increasing chain length. However, P_2 was an exception in the Muscatine and Canisteo soils under aerobic conditions, for which the k_1 values were lower than the k_1 values of P_3. Trimetaphosphate often showed lower k_1 and k_2 values than those of its linear counterpart, P_3. This could be because TMP requires an additional step of opening of the ring structure by an enzyme-catalyzed reaction (i.e., trimetaphosphatase) or by chemically catalyzed reactions (i.e., Ca^{2+}, Mg^{2+}) before cleavage of Pi by polyphosphatases can proceed (Busman and Tabatabai, 1985).

PART III. FACTORS AFFECTING HYDROLYSIS OF POLYPHOSPHATES ADDED TO SOILS

INTRODUCTION

The degree of hydrolysis of polyphosphates added to soils is important in assessing the availability of polyphosphate P for plants because plants mainly take up P as orthophosphate (Pi). The hydrolysis of polyphosphates in soils is the result of chemical and biochemical reactions (Gilliam and Sample, 1968; Hashimoto et al., 1969; Busman and Tabatabai, 1985). These reactions are influenced by complex interactions of many factors. In general, the hydrolysis of polyphosphates is dependent upon temperature, pH, substrate concentration, enzyme concentration, and ionic environment (Van Wazer, 1958).

Pyrophosphate is the most widely studied source of P in polyphosphate fertilizers. Sutton et al. (1966) found a positive relationship between the biological activity (as measured by CO_2 evolution) and the rates of hydrolysis of pyrophosphate in soils. Other factors that have been shown to influence pyrophosphate hydrolysis include soil pH (Sutton and Larsen, 1964; Hossner and Melton, 1970; Blanchar and Hossner, 1969), organic C content, percentage of clay, and the mole fraction of Mg/Mg + Ca (Tabatabai and Dick, 1979).

Evidence for chemical hydrolysis of low-molecular-weight P compounds are derived from studies on steam-sterilized soils. Such studies have shown that from 0 to 77% of the total hydrolysis of such compounds as pyrophosphate, tripolyphosphate, and trimetaphosphate (TMP) could be attributed to chemical

hydrolysis, depending on the soil type and length of incubation period (Gilliam and Sample, 1968; Hashimoto et al., 1969; Dick and Tabatabai, 1978; Busman and Tabatabai, 1985). Other studies have shown that polyphosphates vary in their degree of sorption by soil constituents (Busman, 1984) and that TMP is not sorbed by soils (Blanchar and Hossner, 1969; Busman, 1984). The lack of sorption of TMP by soils is a favorable characteristic for a source of P fertilizer. In Part II, I found that, depending on the type of polyphosphate, type of soil, and soil moisture status, from 37 to 70% of the polyphosphates tested (seven linear oligomers ranging from P_2 to P_{65} and TMP) were hydrolyzed in soils incubated at 25°C for 14 days. Thus, the favorable characteristics in terms of slow release effects of linear polyphosphates or possible leaching of TMP with water to the subsoil is rapidly lost because of the hydrolysis reactions. Consequently, information is needed concerning the effects and interactions of soil properties on polyphosphate hydrolysis. This information would be useful in setting priorities for developing model P fertilizer compounds. Therefore, the objectives of this part of the study were to assess the factors affecting hydrolysis of seven linear oligomers (P_2, P_3, P_5, P_{15}, P_{25}, P_{35}, and P_{65}) and TMP in soils. In this study, I used 29 Iowa surface soils and each were analyzed for 17 chemical and physical properties.

MATERIALS AND METHODS

The polyphosphates used (Table 9) were reagent grade. With the exception of pyrophosphate and tripolyphosphate which were obtained from the Fisher Scientific Co. (Itasca, Illinois), the polyphosphates were obtained from Sigma Chemical Co. (St. Louis, Missouri). The percentage of total P and free Pi in each compound used are reported in Table 1, Part I.

Twenty-nine surface soils (0-15 cm) were selected to give a range of soil properties (Table 10). Field-moist soil samples were collected, and immediately sieved to pass a 5-mm screen and divided into two portions. One portion was placed in a polyethylene bag and stored at 4°C. The second portion was spread on clean paper and air dried at room temperature (22°C). A part of the air-dried sample was ground to pass a 80-mesh sieve, whereas the last was crushed to pass a 2-mm screen. For the soil properties reported in Table 10, pH was determined by a combination glass electrode (soil:water ratio, 1:2.5), organic C by the method of Mebius (1960), total P by the method of Dick and Tabatabai (1977a), and inorganic P as described by Olsen and Dean (1965). Organic P was determined by substracting the value of inorganic P from that obtained for total P. Inorganic C was determined by the method of Bundy and Bremner (1972). The cation-exchange capacity was measured by the method of Chapman (1965). Particle-size

Table 9. Polyphosphate compounds used

Name	Formula	Abbreviation
Sodium pyrophosphate	$Na_4P_2O_7 \cdot 10H_2O$	P_2
Sodium tripolyphosphate	$Na_4P_3O_{10}$	P_3
Sodium trimetaphosphate	$Na_3P_3O_9$	P_3, TMP
Sodium pentapolyphosphate	$Na_7P_5O_{16}$	P_5
Sodium 15-polyphosphate	$Na_{17}P_{15}O_{46}$	P_{15}
Sodium 25-polyphosphate	$Na_{27}P_{25}O_{76}$	P_{25}
Sodium 35-polyphosphate	$Na_{37}P_{35}O_{106}$	P_{35}
Sodium 65-polyphosphate	$Na_{67}P_{65}O_{196}$	P_{65}

distribution by the pipette method of Kilmer and Alexander (1949). Water-soluble Ca^{2+} and water-soluble Mg^{2+} were determined by shaking 2 g of soil with 30 mL of water in a 50-mL plastic centrifuge tube for 30 minutes, followed by centrifuging the suspension (1 min at 10,000 x g), and then determining the Ca^{2+} and Mg^{2+} in the supernatant by atomic absorption spectrophotometry. The method of Jackson (1956) was used to determine dithionite-extractable Fe^{3+} and Al^{3+}, except that atomic absorption spectrophotometry was used for the analysis of Fe^{3+} and Al^{3+}. Specific surface area was determined by the method described by Klein (1981). Buffered pyrophosphatase activity (BPA) was determined by the method of Dick and

Table 10. The ranges and means of selected chemical and physical properties of soils used

Soil property	Chemical and physical properties of soil groups specified			
	Acid soil[a]		Calcareous soils[b]	
	Range	Mean	Range	Mean
pH	5.2-7.2	6.3	7.1-8.00	7.6
Organic C (%)	0.63-3.78	2.04	0.36-5.59	3.09
Clay (%)	4-33	23	20-37	30
Silt (%)	3-78	51	34-71	56
Sand (%)	1-93	26	3-41	15
Specific surface area (m^2/g soil)	15-206	113.6	131-293	201
CEC (cmole (NH_4^+)/kg soil)	7.8-31.9	20.3	5.6-44.5	27.9
Total N (%)	0.06-0.40	0.20	0.04-0.52	0.29
Total P (mg/kg soil)	325-1097	560	653-1293	916
Inorganic P (mg/kg soil)	34-334	179	295-827	499
Organic P (mg/kg soil)	151-763	378	178-846	416
Water-extractable Ca^{2+} (mg/kg soil)	162-45	25	28-72	48
Water-extractable Mg^{2+} (mg/kg soil)	14-57	34	25-52	41
Dithionite-extractable Fe^{3+} (mg/kg soil)	1689-8035	4321	859-6230	3726
Dithionite-extractable Al^{3+} (mg/kg soil)	209-1208	579	187-658	399
Buffered pyrophosphatase activity[c]	54-110	350	43-281	157
Nonbuffered pyrophosphatase activity[c]	84-841	365	77-361	138

[a] 20 soils, Table 25, Appendix.

[b] 9 soils, Table 25, Appendix.

[c] mg Pi released/kg soil/5 h.

Tabatabai (1978), whereas this same method was modified by substituting nonbuffered pyrophosphate solution for buffered pyrophosphate solution (buffering solution replaced by deionized water) to determine the nonbuffered pyrophosphatase activity (NPA). The finely ground soil samples (<80 mesh) were used in the determination of organic C, total N, organic P, inorganic P, and total P, and all other analyses were done on the <2-mm soil samples.

The hydrolysis of the polyphosphates in soils was determined under aerobic conditions. A 2-g soil sample was placed in a 50-ml plastic centrifuge tube and treated with 1 mL of polyphosphate solution containing 1 mg P of a specific compound (500 μg P/g soil). This solution was added dropwise to uniformly moisten the whole soil sample (70% of water-holding capacity). The tube was then stoppered and incubated at 25°C for 7 days. The tube was aerated daily by removing the stopper and flushing the tube with air. The moisture content was maintained by weighing the tubes daily and adding the required amount of water. After incubation, 1 mL of water was added and the orthophosphate (Pi) produced was extracted with 0.5 $\underline{M}$ H_2SO_4 and determined as described by Dick and Tabatabai (1978).

Controls were performed to correct for the native Pi in each soil and for any trace amounts of Pi that might be present in the polyphosphates. For the control, the 2 g of

soil were treated with 1 mL of water and incubated along with the polyphosphate-treated soils. After incubation, 1 mL solution containing 1 mg of the corresponding polyphosphate-P was added to the control, and the Pi was immediately extracted and determined as previously described for the polyphosphate-treated samples.

This same procedure was followed in the study of hydrolysis of polyphosphates in steam-sterilized soil, except that the 50-mL centrifuge tube containing 2 g soil was first autoclaved at 121°C for 1 h. In addition, each polyphosphate solution was cold-sterilized by filtering through a sterile, 0.2-mm Metrical GA-8 membrane (Gelman Sci. Inc., Ann Arbor, Michigan) into sterile suction flasks. A preliminary study was conducted to determine whether sterility could be maintained for a 7-day incuation period in steam-sterilized soils. For this test, six soils with a range in soil properties were selected. A 2-g sample of soil in a 50-mL centrifuge tube was autoclaved and then 1 mL of cold, sterilized deionized water was added and incubated at 25°C. After 7 days, 25 mL sterile, deionized water was added to each tube and placed on an end-to-end shaker for 3 min. A 2-mL aliquot was removed with a sterile pipette and placed in a sterile Petri dish (100 mm x 15 mm) containing sterile growth media (Difco Laboratories, 1953), and incubated at 37°C for 48 h. No microbial growth was noted in any of these treatments after

the 48-h incubation period. In a similar experiment, 1 mL of nonsterile, deionized water was added to the steam-sterilized soil sample instead of sterile water and all of these treatments did show microbial growth upon extraction and incubation of the growth media.

All values reported are averages of duplicate determinations expressed on a moisture-free basis, moisture being determined from weight loss after drying at 105°C for 48 h.

RESULTS AND DISCUSSION

The formulae and chemical composition of the polyphosphates in this study are shown in Table 1, Part I, and Table 9. The P compounds were reagent grade and contained only small amounts of orthophosphate (Pi) (<0.39%). The percentage of P added to the soils (500 µg P/g soil) was designed to approximate that which occurs in a fertilizer band.

There were 29 Iowa surface soils selected to provide a wide range of soil characteristics (Table 10). Seventeen soil parameters were determined on each soil. These factors were chosen because they could potentially affect the hydrolysis of polyphosphates in soils. The factors: pH, organic C content, and total N, would be important in microbial activity, whereas such factors as texture, specific surface area, dithionite-extractable Fe^{3+} and Al^{3+} could be important in sorption reactions of polyphosphates. Calcium and Mg^{2+} were included because they are known to be important in both enzymatic and chemical catalysis of certain polyphosphates (Tabatabai and Dick, 1979; Busman and Tabatabai, 1985; Healy and Kilpatrick, 1955). The P fractions of organic P and inorganic P plus total P were included because of their potential effect on the amount of native orthophosphate (Pi in soil solution). This could be of importance because Pi is known to inhibit phosphatase activity (Kulaev, 1979), and could also affect equilibrium relationships in chemical hydrolysis of

polyphosphates because the ultimate reaction product is Pi. Pyrophosphatase activity was also measured on the soils to compare this short-term (5 h) enzyme assay with a 7-day incubation and to determine whether pyrophosphatase activity could be useful for predicting hydrolysis of polyphosphates.

Simple Regression Analyses

Polyphosphates can be hydrolyzed by both chemical and enzymatic reactions, and a given soil property may affect these two mechanisms in a different manner. Consequently, this study included determination of the amount of Pi produced in hydrolysis of polyphosphates in steam-sterilized soils to characterize the chemical hydrolysis, and in hydrolysis of polyphosphates in nonsterile soils to characterize the total Pi produced from chemical and biochemical reactions.

The amounts of Pi produced from polyphosphate hydrolysis varied among the soils (Table 11). For example, the amounts of Pi produced from P_2 ranged from 84 to 335 and from 2 to 186 μg/g soil for total hydrolysis (chemical and biochemical) and for chemical hydrolysis, respectively. From the results obtained (Table 11), it is evident that chemical hydrolysis is an important pathway for the conversion of the polyphosphates to Pi, because an average of 27% (P_2) and from 40 to 47% (P_3, TMP, P_5, P_{15}, P_{25}, P_{35}, and P_{65}) of the total hydrolysis could be attributed to chemical hydrolysis.

Table 11. The ranges, means and standard deviations (SD) of the amounts of Pi produced from polyphosphates added to soils and incubated under nonsterile or sterile conditions for 7 days at 25°C

P compound	Amount of Pi produced for soil group specified[a]					
	Nonsterile			Steam-sterilized[b]		
	Range	Mean	SD	Range	Mean	SD
	mg Pi/kg soil/7 days					
P_2	84-335	211	59	2-186	58	45
P_3	114-359	211	65	29-186	94	31
TMP	102-330	190	62	22-210	86	40
P_5	105-369	197	61	39-227	94	39
P_{15}	94-310	186	57	13-221	85	43
P_{25}	89-277	177	61	20-200	74	38
P_{35}	80-287	169	61	27-186	68	35
P_{65}	81-279	151	58	17-180	68	36

[a]Tables 26 and 27, Appendix.

[b]Autoclaved 1 h at 121°C.

Regression analysis shows that the amounts of Pi produced from the polyphosphates correlated with very few soil properties (Table 12). With the exception of P_2, which showed a nonsignificant r value, polyphosphate hydrolysis was positively correlated with pH. This is consistent with previous findings on the effect of soil pH on hydrolysis of pyrophosphate (Sutton and Larsen, 1964; also see Part II).

Table 12. Simple correlation coefficients (r) for paired relationships between the amounts of Pi produced from hydrolysis of polyphosphates added to soils and selected properties of Iowa surface soils

Soil property	Correlation coefficient		
	P_2	P_3	TMP
pH	0.25	0.47**	0.70**
Inorganic P	-0.05	0.10	0.25
Water-extractable Ca^{2+}	0.09	0.25	0.33
Dithionite-extractable Fe^{3+}	-0.30	-0.49*	-0.38*
Dithionite-extractable Al^{3+}	-0.42*	-0.56*	-0.58**
BPA[a]	-0.20	-0.36	-0.36
NPA[b]	-0.10	-0.13	-0.35

[a]BPA, buffered pyrophosphatase activity.

[b]NPA, nonbuffered pyrophosphatase activity.

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

***Significant at 0.001 probability level.

(r) for polyphosphate specified				
P_5	P_{15}	P_{25}	P_{35}	P_{65}
0.57**	0.65***	0.70***	0.72***	0.66***
0.15	0.28	0.37*	0.36*	0.28
0.26	0.35	0.55	0.52*	0.39*
-0.46*	-0.37*	-0.42*	-0.39*	-0.51**
-0.65**	-0.62***	-0.62***	-0.64***	-0.68***
-0.37*	-0.47**	-0.51**	-0.49**	-0.37**
-0.34	-0.44*	-0.52**	-0.53**	-0.36**

Regression equations of the plots made of the amount of Pi produced from each polyphosphate against soil pH showed similar slopes (except for P_2), indicating that the effect of pH on hydrolysis of the P compound is uniform (Fig. 8).

Polyphosphate hydrolysis was negatively correlated with dithionite-extractable Fe^{3+} and Al^{3+} (Table 12). This negative effect of Fe^{3+} and Al^{3+} oxides is not surprising because it is known that linear polyphosphates (P_2-P_{65}) are sorbed by soils (Busman, 1984) and that pyrophosphate reacts with Fe and Al in soil solution (Philen and Lehr, 1967; Hashimoto et al., 1969). Dithionite-extractable Al^{3+} is more important than Fe^{3+} in decreasing polyphosphate hydrolysis in soils, which is evident by the correlation coefficient values (Table 12). Except for P_2, all other oligomers showed similar slopes for the linear relationships between the amounts of Pi produced from the polyphosphates and dithionite-extractable Al^{3+} (Fig. 9).

Regression analysis of the amounts of Pi produced from chemical hydrolysis of polyphosphates and 17 soil properties showed very few significant simple correlations (Table 13) as compared with those obtained for total hydrolysis (Table 12). In general, hydrolysis of longer oligomers ($P > 15$) showed greater significant simple correlation coefficients with soil properties than that obtained with the short oligomers ($P < 5$). The chemical hydrolysis of P_{65} was

Figure 8. Relationship between the amounts of Pi produced from hydrolysis of polyphosphates added to soils and the pH of soils

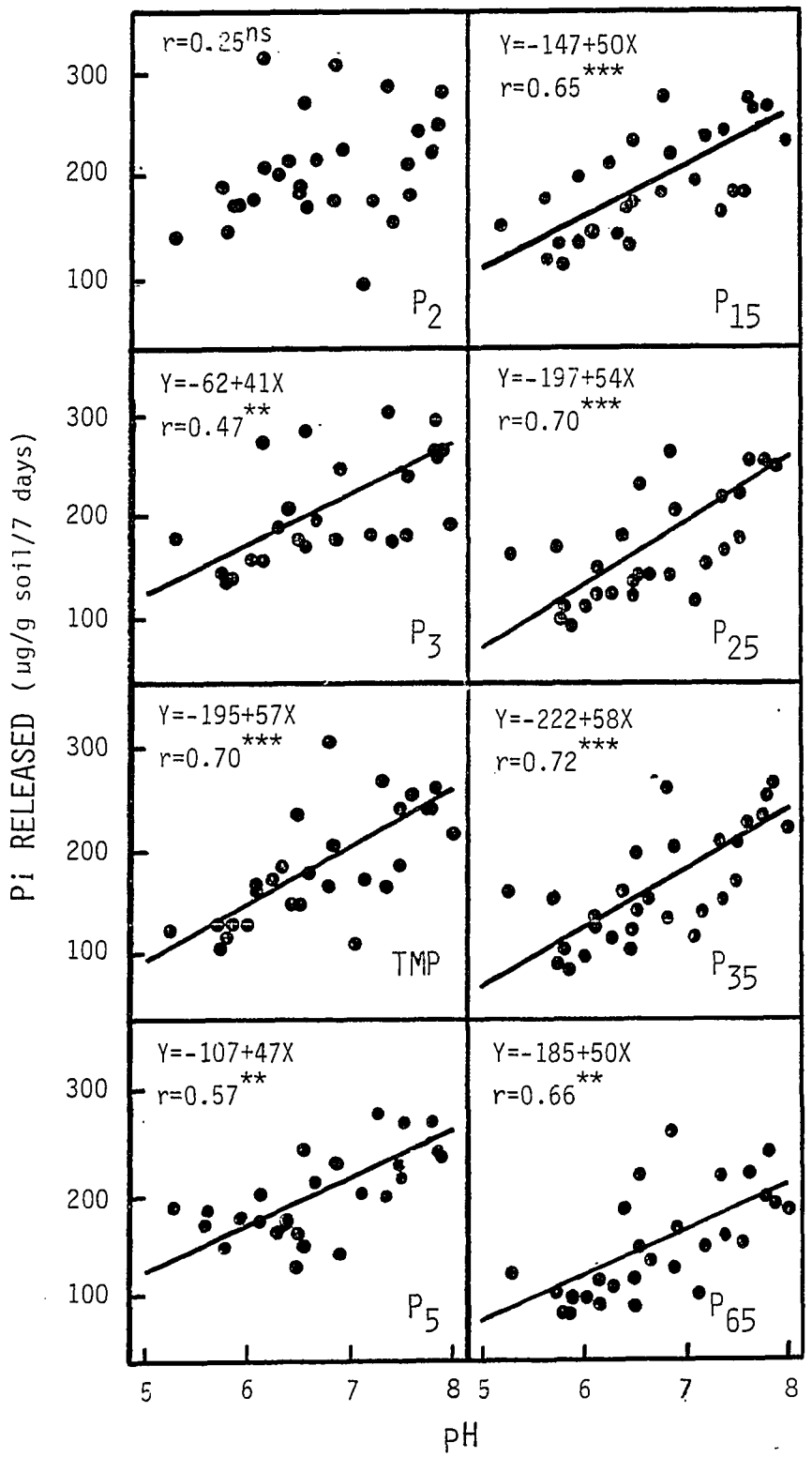

r=0.25ns
P2
Y=-147+50X
r=0.65***
P15
Y=-62+41X
r=0.47**
P3
Y=-197+54X
r=0.70***
P25
Y=-195+57X
r=0.70***
TMP
Y=-222+58X
r=0.72***
P35
Y=-107+47X
r=0.57**
P5
Y=-185+50X
r=0.66**
P65
Pi RELEASED (µg/g soil/7 days)
100
200
300
5
6
7
8
pH

Figure 9. Relationship between the amounts of Pi produced for hydrolysis of polyphosphates added to soils and dithionite-extractable Al^{3+} in soils

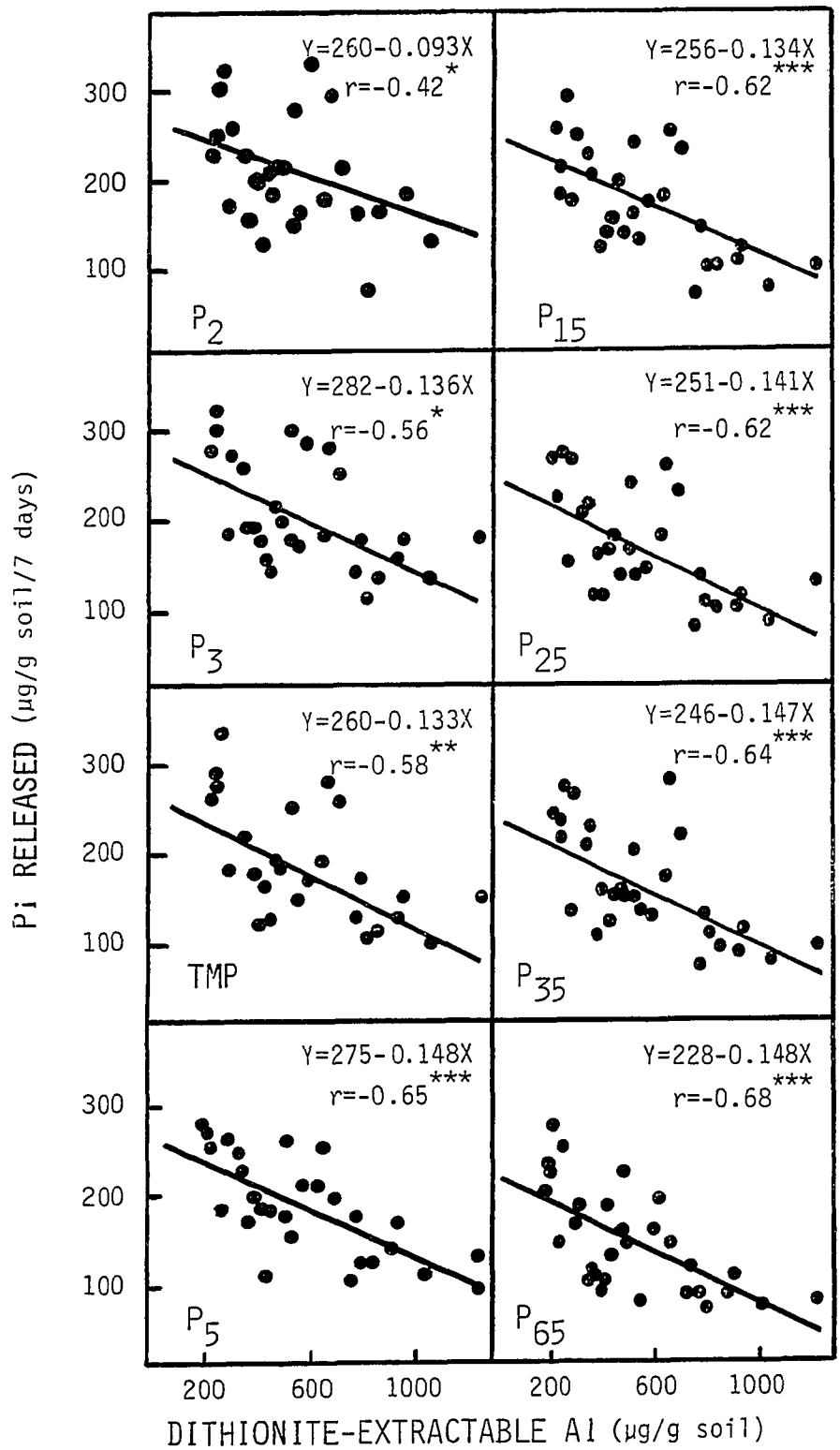

Y=260-0.093X
r=-0.42*
P2
Y=256-0.134X
r=-0.62***
P15
Y=282-0.136X
r=-0.56*
P3
Y=251-0.141X
r=-0.62***
P25
Y=260-0.133X
r=-0.58**
TMP
Y=246-0.147X
r=-0.64***
P35
Y=275-0.148X
r=-0.65***
P5
Y=228-0.148X
r=-0.68***
P65
300
200
100
200
600
1000
Pi RELEASED (µg/g soil/7 days)
DITHIONITE-EXTRACTABLE Al (µg/g soil)

Table 13. Simple correlation coefficients (r) for paired relationships between the amounts of Pi produced from chemical hydrolysis of polyphosphate added to soils and selected properties of Iowa surface soils

Soil property	Correlation coefficient		
	P_2	P_3	TMP
pH	-0.18	0.05	0.31
Organic C	0.19	0.28	0.26
Specific surface area	0.04	0.22	0.38
CEC	0.23	0.44	0.36
Total P	0.08	0.13	0.22
Inorganic P	-0.02	-0.06	0.14
Organic P	0.14	0.27	0.15
Water-extractable Ca^{2+}	0.20	0.40*	0.36
Dithionite-extractable Fe^{3+}	-0.25	-0.21	-0.37*
Dithionite-extractable Al^{3+}	-0.16	-0.09	-0.39*

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

***Significant at 0.001 probability level.

(r) for polyphosphate specified				
P_5	P_{15}	P_{25}	P_{35}	P_{65}
0.38	0.35	0.41*	0.41*	0.62***
0.17	0.38*	0.25	0.07	0.12
0.30	0.47**	0.42*	0.23	0.31
0.15	0.36	0.37*	0.13	0.04
0.43*	0.60***	0.43*	0.34	0.60***
0.19	0.32	0.39*	0.08	0.37*
0.42*	0.52**	0.22	0.41*	0.48*
0.29	0.53**	0.38*	0.34	0.67***
-0.24	-0.16	-0.08	-0.07	-0.17
-0.28	-0.15	-0.14	-0.01	-0.29

significantly correlated with pH, total P, inorganic P, organic P, and water-extractable Ca^{2+}.

Multiple Regression Models

Since hydrolysis reactions of polyphosphates in soils is the result of complex interactions of various factors, it is not unexpected that simple correlations would be limited in describing the relationship between hydrolysis rates and soil properties. Consequently, multiple regression models were developed to predict the amount of Pi that would be produced (dependent variable Y) in a 7-day period. Through this modeling process or factor analysis, those soil properties which made significant contributions to the model could be identified as being important in determining polyphosphate hydrolysis in soils.

As a preliminary step in developing the models, simple correlations and intercorrelations (latent roots procedure, Gunst et al., 1976) of the variables were examined. A high degree of intercorrelation can cause distortion of the regression model and thus complicate variable selection. This is particularly important in soils where soil properties are often correlated. Criteria for determining whether there were severe intercorrelations were if the r value for two independent variables was >0.60 (Henao, 1976) or for a group of variables where the latent root was <0.3 (Gunst et al., 1976).

Factors found to have significant intercorrelations fall into three groups: (1) clay, silt, sand, and specific surface area; (2) total P, inorganic P, and organic P; and (3) buffered pyrophosphatase activity and nonbuffered pyrophosphatase activity. Because it is likely that all the variables in a given category would have a similar effect on the hydrolysis reactions, each variable for a group was tested in alternative models (one included and the other deleted), and the variable with the higher regression R^2-value was the one retained. However, there were other intercorrelations where the variables would not likely have the same effect on polyphosphate hydrolysis. This was true of pH and organic C, which were both intercorrelated with clay content, specific surface area, and total N. For example, pH would affect the hydrolysis reaction by affecting enzymatic activity (e.g., ionization states of enzymes and substrates) while clay content would affect hydrolysis activity by affecting sorption of polyphosphates. Consequently, these variables were not considered to be mutually exclusive and were retained for preliminary regression analysis.

Statistical models were developed by using the Statistical Analysis System (Barr et al., 1976) computer language. The multiple regression models were fitted by using the PROC STEPWISE subprogram. After models that maximized the R^2-value were developed, the t-test for significance was applied to each

partial regression coefficient, and the variates with probability levels <0.05 were then retained in the final model.

Chemical hydrolysis models

Development of multiple regression models for predicting chemical polyphosphate hydrolysis in soils was not successful. Various combinations of the variables such as ratios, linear x linear terms, squared terms, etc., and log transformations of both the dependent variable and independent variables (including hydrogen ion concentration) were tested. However, R^2-terms never exceeded 0.45, and were often <0.40. Furthermore, t-tests of the partial correlation coefficients for the variables included in these models showed the probability level was >0.10. The one variable that did come through consistently in the models was inorganic P content. This suggests that the amount of inorganic P in soil solution may be important in regulating chemical hydrolysis of polyphosphates in soils through equilibrium relationships of Pi and polyphosphate concentrations in soils. These results suggest that use of steam-sterilized soils may be of limited usefulness in studies to predict chemical hydrolysis of polyphosphates.

Total hydrolysis models

Table 14 shows the final regression models for the total hydrolysis of the polyphosphates added to soils. The variable pH again showed a positive partial correlation coefficient

Table 14. Multiple regression equations and coefficients of determination (R^2) for relationships between the amounts of Pi produced from hydrolysis of polyphosphates added to soils and selected properties of Iowa surface soils

P compound	Regression equation[a]	R^2[b]
P_2	Y[c] = 197 + 19(pH) - 4.9(cl)[d] + 3.4(Ca) + 0.209(NPA)[e] - 3.7(Mg) - 0.061(Al)	0.66
P_3	Y = 52 + 39(pH) - 7.9(cl) + 2.8(Ca) + 0.136(NPA) - 2.5(Mg) + 22(OC)[f]	0.77
TMP	Y = -145 + 61(pH) - 6.2(cl) + 2.2(Ca) + 0.137(NPA) - 1.8(Mg) + 16(OC)	0.84
P_5	Y = 39 + 40(pH) - 3.7(cl) + 1.8(Ca) + 0.112(NPA) - 1.5(Mg) - 0.091(Al)	0.72
P_{15}	Y = -5.6 + 42(pH) - 3.6(cl) + 1.8(Ca) + 0.083(NPA) - 1.3(Mg) - 0.066(Al)	0.76
P_{25}	Y = -23 + 40(pH) - 3.9(cl) + 3.0(Ca) + 0.084(NPA) - 1.6(Mg) + 0.057(Al)	0.88
P_{35}	Y = -89 + 47(pH) - 4.0(cl) + 1.9(Ca) + 0.046(NPA) - 0.052(Al)	0.84
P_{65}	Y = -99 + 46(pH) - 3.0(Cl) + 1.1(Ca) + 0.090(NPA) - 0.082(Al)	0.75

[a] All regression coefficients were significant at 0.05 probability level.

[b] All R^2 values were significant at 0.001 probability level.

[c] Y = μg P/g soil/7 days.

[d] cl, clay content (%).

[e] NPA, nonbuffered pyrophosphatase activity.

[f] OC, organic carbon (%).

which is consistent with the simple correlations reported above. Substituting hydrogen ion concentration for pH in the models resulted in R^2 values being less than or equal to R^2 values in models containing pH. Clay content consistently showed negative partial correlation coefficients, which indicates it is inhibiting polyphosphate hydrolysis in soils. Clay minerals may decrease rates of hydrolysis through sorption reactions of polyphosphates (Busman, 1984).

Water-soluble Ca^{2+} was a significant factor in catalyzing polyphosphates hydrolysis in soils, which was evident from the positive partial correlation coefficients. This indicates that Ca^{2+} is involved in chemical hydrolysis since trimetaphosphate has been shown to be activated by Ca^{2+} in sterile water (Healy and Kilpatrick, 1955) and in steam-sterilized soils (Busman and Tabatabai, 1985), whereas, in the case of pyrophosphatase activity, Ca^{2+} has been shown to inhibit catalysis of pyrophosphate (Searle and Hughes, 1977; Tabatabai and Dick, 1979).

Water-soluble Mg^{2+} showed a negative partial correlation coefficient with hydrolysis of TMP and oligomers $< p_{25}$. This is contrary to previous studies on the role of Mg^{2+} in hydrolysis reactions of pyrophosphate and TMP. The Mg^{2+} cation is required for activation of pyrophosphate (the active substrate) in pyrophosphatase assay (Searle and Hughes, 1977) and has been shown to promote chemical hydrolysis of TMP in soils

(Busman and Tabatabai, 1985). Tabatabai and Dick (1979) found that logarithms of pyrophosphatase activity was negatively correlated ($r = -0.78^{**}$) with the water-soluble mole fraction Mg/(Mg + Ca). In the present study, simple correlations or inclusion of this variable in the multiple regression model did not show significant relationship between the Mg/(Mg + Ca) mole fraction and polyphosphate hydrolysis. All of the studies cited above were of short duration, ranging from a few minutes to 5 h, whereas, in the present study, we used an incubation period of 7 days. Consequently, in a longer incubation period, the net effect of Mg^{2+} appears to be inhibiting polyphosphate hydrolysis, possibly by some complexation reaction. Other ratios of Ca^{2+} and Mg^{2+} together or in combination with Al^{3+} and Fe^{3+} plus various linear x linear terms or squared terms were included in the model development, but again, none of these made significant contributions to the model.

Dithionite-extractable Al^{3+} showed a negative partial correlation coefficient for P_2, P_5, P_{15}, P_{25}, P_{35}, and P_{65} (Table 14). This corroborates with results of the simple correlations reported above and gives evidence that polyphosphate hydrolysis is inhibited by Al^{3+}. This could be due to the formation alumno-polyphosphate reactions products which reduces the susceptibility of polyphosphates for hydrolysis. Another role of Al^{3+} could be in inhibiting phosphatase

activity (Searle and Hughs, 1971). However, in the case of P_3 and TMP, dithionite-extractable Al^{3+} did not make a significant contribution to the model. In the case of TMP, this could be expected because TMP has been shown not to be sorbed by soil constituents (Blanchar and Hossner, 1969; Busman, 1984) and thus Al reaction products would not form with TMP.

Organic C would be expected to be important with regards to energy relationships in microbial phosphatase synthesis. However, only with P_3 and TMP did the partial correlation coefficients of organic C content make a significant contribution to the model.

Nonbuffered pyrophosphatase activity (NPA) was a much more important predictor variable than buffered pyrophosphatase activity (BPA). This occurred because the polyphosphates in the present study were incubated for 7 days in a nonbuffered system, which would be better correlated with NPA. It is interesting to note that NPA and polyphosphate hydrolysis were negatively but poorly correlated in simple regressions (Table 12). However, NPA made a significant contribution to the multiple regression model and had the expected positive partial correlation coefficient. This observation reinforces the concept that polyphosphate hydrolysis is the result of complex interactions of several soil properties and that simple correlations are limited in providing information on the soil factors affecting hydrolysis of polyphosphates.

Table 15 shows the standardized partial regression coefficients (Steel and Torrie, 1980) derived from the partial regression coefficients of Table 14. Standardized partial regression coefficients can be used to rank or give the relative importance of each variable in its contribution to the regression model. In general, pH (except for P_2), clay content, and Ca^{2+} (except for P_{65}) were relatively important, whereas Mg^{2+}, Al^{3+}, organic C contents, and NPA were less important in predicting polyphosphate hydrolysis in soils.

To determine how well the models fit, a final investigation of the residuals was conducted. Since all the sample information on lack of fit is contained in the residuals, plotting residuals in various ways can be used to identify anomolies in the model (Johnson and Wichern, 1982). For each polyphosphate model, plots of the residuals against predicted values and plots of the residuals against a predictor variable were made. All the plots were randomly distributed and no systematic pattern or trend was noted. This indicates that the models were providing a good fit and that they should be good predictors of polyphosphate hydrolysis in soils.

Table 15. Standardized partial regression coefficients derived from multiple regression equations predicting polyphosphate hydrolysis in soils

P compound	Standardized partial regression coefficients[a]					
P_2	0.88(Ca)	-0.66(Mg)	00.54(NPA)	-0.62(cl)	-0.27(Al)	0.24(pH)
P_3	-0.93(cl)[b]	0.68(Ca)	0.46(pH)	-0.41(Mg)	0.40(OC)[c]	0.39(NPA)
TMP	-0.76(cl)	0.74(pH)	0.56(Ca)	0.41(NPA)[d]	-0.31(Mg)	0.29(OC)
P_5	0.49(pH)	-0.47(cl)	0.45(Ca)	-0.40(Al)	0.35(NPA)	-0.27(Mg)
P_{15}	0.54(pH)	0.50(Ca)	-0.47(cl)	-0.30(Al)	0.27(NPA)	-0.25(Mg)
P_{25}	0.77(Ca)	0.49(pH)	-0.48(cl)	-0.29(Mg)	0.26(NPA)	0.24(Al)
P_{35}	0.57(pH)	-0.49(cl)	0.47(Ca)	-0.22(Al)	0.14(NPA)	
P_{65}	0.59(pH)	-0.40(cl)	-0.37(Al)	0.32(Ca)	0.29(NPA)	

[a]Standardized partial regression coefficient = $b_i \frac{Si}{Sy}$; where b_i is the partial regression coefficient (regression of Y on X_i for fixed values of other Xs). S_i = standard deviation of b_i, and S_y = standard deviation of Y.

[b]cl, clay content (%).

[c]OC, organic carbon (%).

[d]NPA, nonbuffered pyrophosphatase activity.

PART IV. POLYPHOSPHATES AS SOURCES OF PHOSPHORUS FOR PLANTS

INTRODUCTION

Condensed inorganic phosphates (polyphosphates) are of interest as P fertilizer sources because of their water solubility, high P content, and their potential as micronutrient carriers. Ammonium polyphosphates are widely available for commercial use and so-called metaphosphates have been tested in the past as sources of P for plants. Numerous greenhouse and field studies have shown that these polyphosphates provide adequate amounts of P to plants and compare favorably with conventional P fertilizers (Englestad and Terman, 1980). These fertilizer materials, however, are ill-defined and contain a mixture of P compounds including orthophosphate (P_1). Other specific polyphosphates exhibit characteristics that may be useful in developing more efficient P fertilizers. In Part II, I found that long, linear oligomers (e.g., P_{65}) have significantly lower rates of hydrolysis in soils than short oligomers (e.g., pyrophosphates or tripolyphosphates) when incubated up to 14 days at 25°C. These results suggested that long-linear oligomers may be useful as slow-release P sources, since plants largely take up P as orthophosphate (Pi), which is the end product of polyphosphate hydrolysis. Furthermore, trimetaphosphate (TMP) has the unique characteristic of not being sorbed by soils (Blanchar and Hossner, 1969; Busman, 1984), which is a favorable attribute, because P availability is largely limited by

sorption reactions in soils.

With the exception of pyrophosphate which has usually been shown to be equally available to Pi in terms of P uptake by plants (Blanchar and Hossner, 1969; Englestad and Allen, 1971; Hughes and Hashimoto, 1971), little information is available concerning the availability to plants of TMP and long, linear oligomers of known structures. Consequently, greenhouse studies were conducted to compare the effect of sodium phosphate (P_1), four linear oligomers (P_2, P_3, P_{15}, and P_{45}), and one cyclic polyphosphate (TMP) on the dry matter yield and P uptake by plants.

MATERIALS AND METHODS

The soils used (Table 16) were surface soils (0-15 cm) selected to give a range in chemical and physical properties, and were collected from fields that had not received P fertilizers for at least 15 years and had low amounts of plant-available P as evident by the Bray-Kurtz I and Olsen extractants. The field-moist soils were passed through a 1.5-cm screen, mixed thoroughly, and placed in polyethylene bags and stored in a refrigerator at 4°C for five days before use in the greenhouse. A subsample of each soil was air-dried and ground to pass a 2-mm sieve and a portion of it was ground to pass an 80-mesh sieve.

In the analyses reported in Table 16, pH was determined by a glass electrode (soil/water ratio 1:1), organic C by the method of Mebius (1960), total N by the semi-micro-Kjeldahl method described by Bremner and Mulvaney (1982), and inorganic C by the method of Bundy and Bremner (1972). Plant-available P was determined on 1 g soil (on an oven-dry basis) with Bray-Kurtz I reagent on the three acid soils (Clarion, Webster and Primghar) and with Olsen's reagent on the calcareous Ida soil as outlined by Knudsen (1980). Cation-exchange capacity was determined by using neutral 1 $\underline{M}$ NH_4OAc as described by Chapman (1965), and particle-size distribution by the pipette method of Kilmer and Alexander (1949). Inorganic P and organic P were determined by the method of Olsen and Dean (1965), as

Table 16. Properties of soils used

Soil[a]	pH	Organic carbon	Total N	$CaCO_3$ equiv.
		----------%----------		
Clarion	5.4	3.49	0.291	0
Webster	5.9	3.73	0.282	0
Primghar	6.1	3.23	0.274	0
Ida	7.5	1.40	0.145	1.16

[a]Clarion: fine-loamy, mixed, mesic Typic Hapludoll; Webster: fine-loamy, mixed, mesic Typic Haplaquoll; Primghar: fine-silty, mixed, mesic Hapludoll; Ida: fine-silty, mixed (calcareous), mesic Typic Udorthent.

[b]Clarion, Webster, and Primghar were extracted with Bray-Kurtz I solution, whereas Ida was extracted with Olsen's reagent.

[c]cmole (NH_4^+)/kg soil.

Phosphorus							
Total	Org.	Inorg.	Avail.[b]	CEC[c]	Clay	Sand	Moisture
-------mg/kg soil----------					----------%----------		
459	293	166	6.5	24.2	23.4	27.9	19
451	257	194	6.2	34.6	33.7	29.7	20
431	314	116	1.9	34.7	33.0	2.4	21
825	57	768	7.0	19.8	15.5	7.2	18

modified by Chae and Tabatabai (1981), and total P by the method of Dick and Tabatabai (1977a). Organic C, total P, and total N were determined on samples ground to pass an 80-mesh sieve. All other analyses were carried out on the <2-mm soil samples. All results reported are expressed on a moisture-free basis, moisture being determined from loss in weight after drying at 105°C for 48 hours.

Greenhouse Experiments

In the study with ryegrass (Lolium multiflorum Lam) as indicator plants, I used pots (16.5 x 16.5 cm) lined with double polyethylene bags. Each pot contained 1.8 kg (on an oven-dry basis) of field-moist soil. The soils of all pots were equally treated with 79 mg N/kg as NH_4NO_3, 134 mg K/kg as K_2SO_4, and the sulfate salts (71 mg S/kg) of Mg (8 mg Mg/kg), Fe (8 mg Fe/kg), Mn (7 mg Mn/kg), Zn (7 mg Zn/kg), and Cu (2.4 mg Cu/kg).

All sources of P were applied at three levels: 20, 40, and 80 mg P/kg soil. Control treatments were included for each soil. The P compounds used (Table 17) were reagent grade. Trimetaphosphate (TMP), P_{15}, and P_{45} were obtained from Sigma Chemical Co. (St. Louis, Missouri), whereas orthophosphate (P_1), pyrophosphate (P_2), and tripolyphosphate (P_3) were obtained from Fisher Scientific Co. (Itasca, Illinois). The free orthophosphate content and total P of each polyphosphate

Table 17. Phosphorus compounds used

P compound			Total P (%)		Free PO_4^{3-}-P (%)
Name	Formula	Abbreviation	Calculated[a]	Determined	
Sodium phosphate	$NaH_2PO_4 \cdot H_2O$	P_1	22.4	22.2	99.86
Sodium pyrophosphate	$Na_4P_2O_7 \cdot 10H_2O$	P_2	13.9	13.7	0.10
Sodium tripolyphosphate	$Na_4P_3O_{10}$	P_3	25.3	23.6	0.16
Sodium trimetaphosphate	$Na_3P_3O_9$	P_3,TMP	30.4	29.4	0.29
Sodium 15-polyphosphate	$Na_{17}P_{15}O_{46}$	P_{15}	29.2	28.2	0.05
Sodium 45-polyphosphate	$Na_{47}P_{45}O_{136}$	P_{45}	30.0	29.7	0.01

[a]Calculated from the molecular formula.

were determined as described in Part I.

Nitrogen, K, Mg, S, and the micronutrients were applied as a single nutrient solution and the P compounds were applied in a separate solution. These solutions were applied on an individual-pot basis and mixed thoroughly with the soil before potting. One day after the addition of the fertilizers, 1 g of seeds of annual ryegrass was planted in each pot. The seeds germinated well, and a good stand was established.

The pots were watered daily with a volume of deionized water equal to that lost during the previous 24-h period, as determined by weighing 10 randomly chosen pots from each block. The available moisture level was maintained approximately at 0.03 MPa. Once a week all pots were adjusted to a selected weight and rerandomized within the blocks.

The plants were cut at a height of 2 cm from the soil surface every 30 days for a total of four cuttings. After each cutting, all pots received a supplementary 50 mL nutrient solution which provided 92 mg N/kg soil and, for the second cutting, this solution also contained 67 mg K and 28 mg S/kg soil. The lighting in the greenhouse was maintained at 14 h/day and the daily temperatures ranged from 22 to 30°C. After each cutting, the plant material was dried at 65°C, weighed, and ground to pass a 40-mesh sieve.

The design of the experiment was a 6 x 3 x 4 factorial with six P compounds, three P rates plus six control treat-

ments (without applied P), and four soils. The repeated cuttings were considered to be a split plot in time. The layout was a randomized complete-block design with three replications.

In the experiment with corn (Zea mays), the soils from the control pots of the ryegrass experiment were removed after the fourth cutting, sieved to remove the ryegrass roots, and mixed thoroughly. A 150-g sample of soil was then placed in a pot constructed from a PVC pipe (14 x 5 cm) with a sealed base of square plexiglass (8 x 8 cm). Three corn seeds were planted 2 cm below the soil surface. Most pots had a 100% germination rate and the few pots with < 100% germination were replanted to maintain 3 plants/pot.

All procedures of nutrient and P applications and watering were exactly the same as outlined for the ryegrass experiments except that N had a split application of $Ca(NO_3)_2$ at the rate of 79 mg N/kg soil and 100 mg N/kg soil at 0 and 14 days, respectively. This provided a total of 38 mg Ca/kg soil. The plants were harvested at the soil surface and dried and processed as outlined for the ryegrass experiment.

Plant Analysis

Total P and K in the plant material were determined by the heteropoly blue method of Murphy and Riley (1962) and flame photometry, respectively, after digestion with HNO_3

and $HClO_4$ as described by Sommers and Nelson (1972). Total N (including NO_3^-) was determined by the method of Nelson and Sommers (1973).

RESULTS AND DISCUSSION

Because the soils were treated with uniform, heavy application of N and K, the plant samples contained sufficiently high concentrations of these elements. For ryegrass, the total N and K values ranged from 1.6 to 1.8% (avg = 1.7%) and from 1.4 to 1.5% (avg = 1.44%). The percentages of total N and K in the ryegrass and corn dry matter produced with the various P sources were similar; therefore, the results obtained for N and K will not be discussed.

Yield of Dry Matter

The cumulative dry matter yields of ryegrass produced at each application rate of all P sources on Clarion, Webster, Primghar, and Ida soils are shown in Figures 10, 11, 12, and 13, respectively. The yield response curves were similar among the P compounds at each P rate for each soil. The cumulative yield of ryegrass obtained on the control pots of Primghar soil tended to be lower than those obtained with the other three soils.

Although all the soils tested were low in available P prior to the experiment, only Primghar soil showed a substantial increase in dry matter yields of the treated pots compared with the control pots. Nevertheless, analysis of variance (ANOVA) showed that there was a significant main effect of rate of P application (Table 18), indicating that there was

Figure 10. Comparison of cumulative dry matter yields of four cuttings of ryegrass produced on Clarion soil treated with various sources of P at three rates

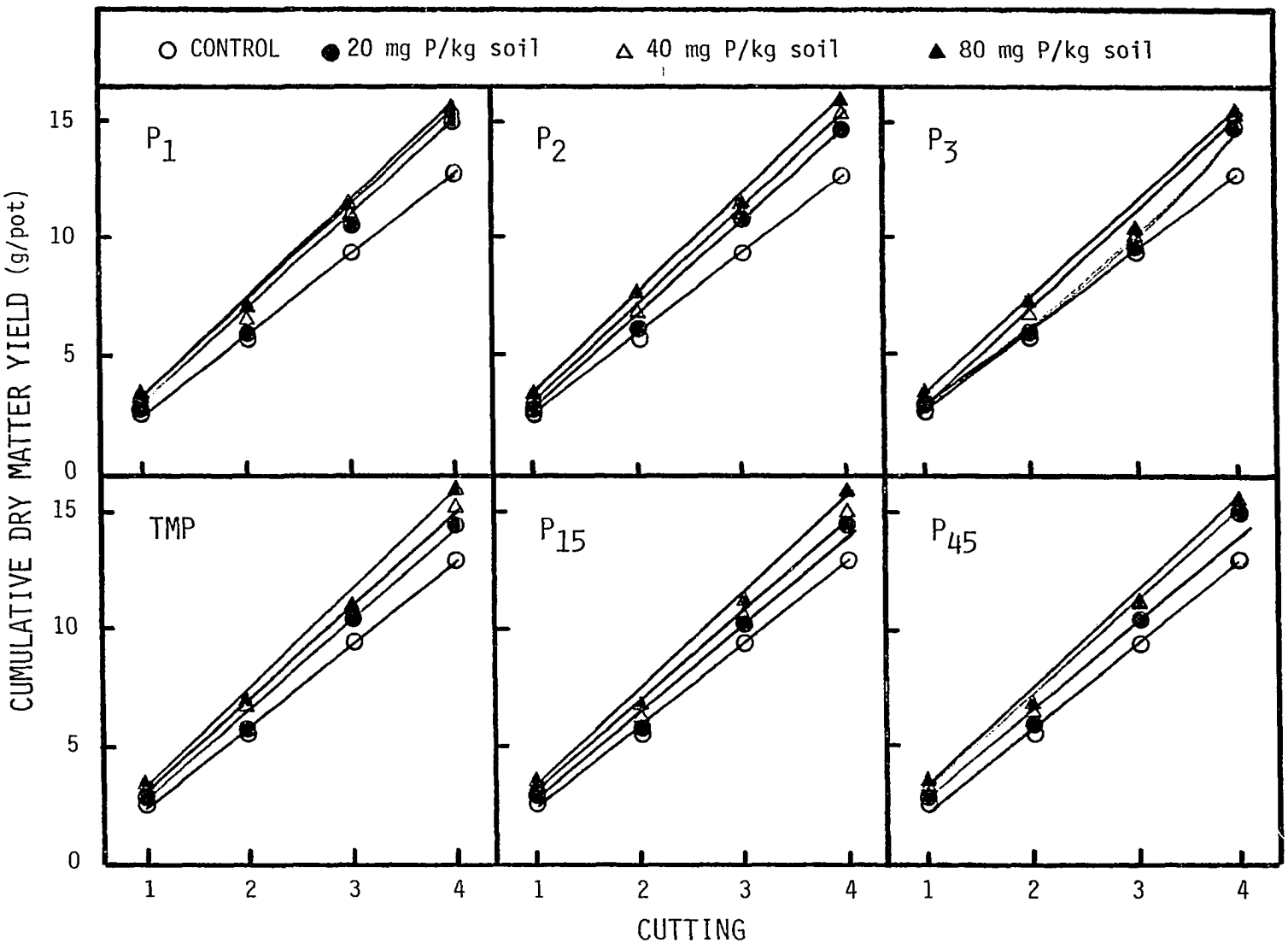

○ CONTROL
● 20 mg P/kg soil
△ 40 mg P/kg soil
▲ 80 mg P/kg soil
P1
P2
P3
TMP
P15
P45
CUMULATIVE DRY MATTER YIELD (g/pot)
0
5
10
15
1
2
3
4
CUTTING

Figure 11. Comparison of cumulative dry matter yields of four cuttings of ryegrass produced on Webster soil treated with various sources of P applied at three rates

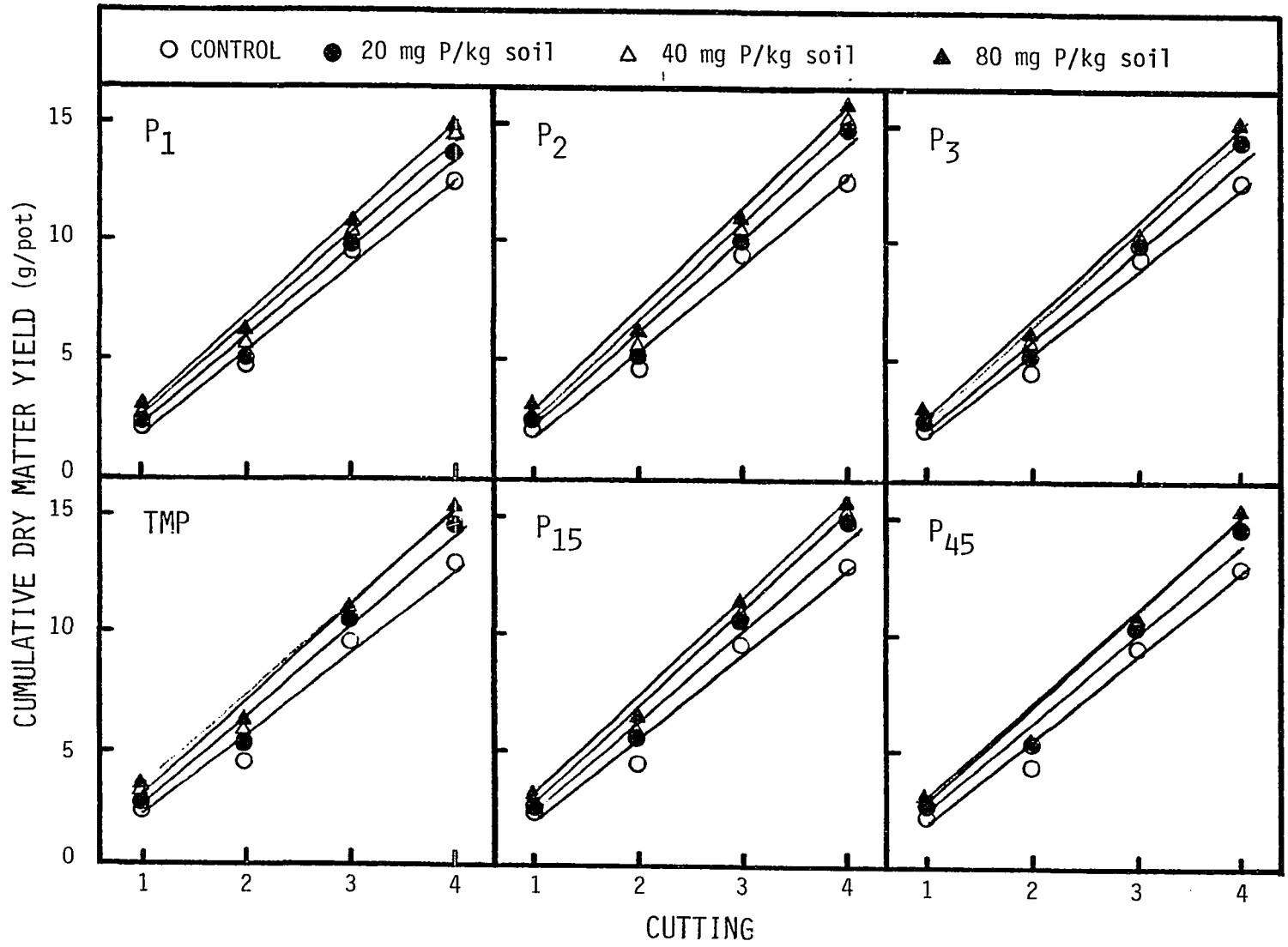

O CONTROL
20 mg P/kg soil
40 mg P/kg soil
80 mg P/kg soil
P1
P2
P3
TMP
P15
P45
CUMULATIVE DRY MATTER YIELD (g/pot)
0
5
10
15
1
2
3
4
CUTTING

Figure 12. Comparison of cumulative dry matter yields of four cuttings of ryegrass produced on Primghar soil treated with various sources of P applied at three rates

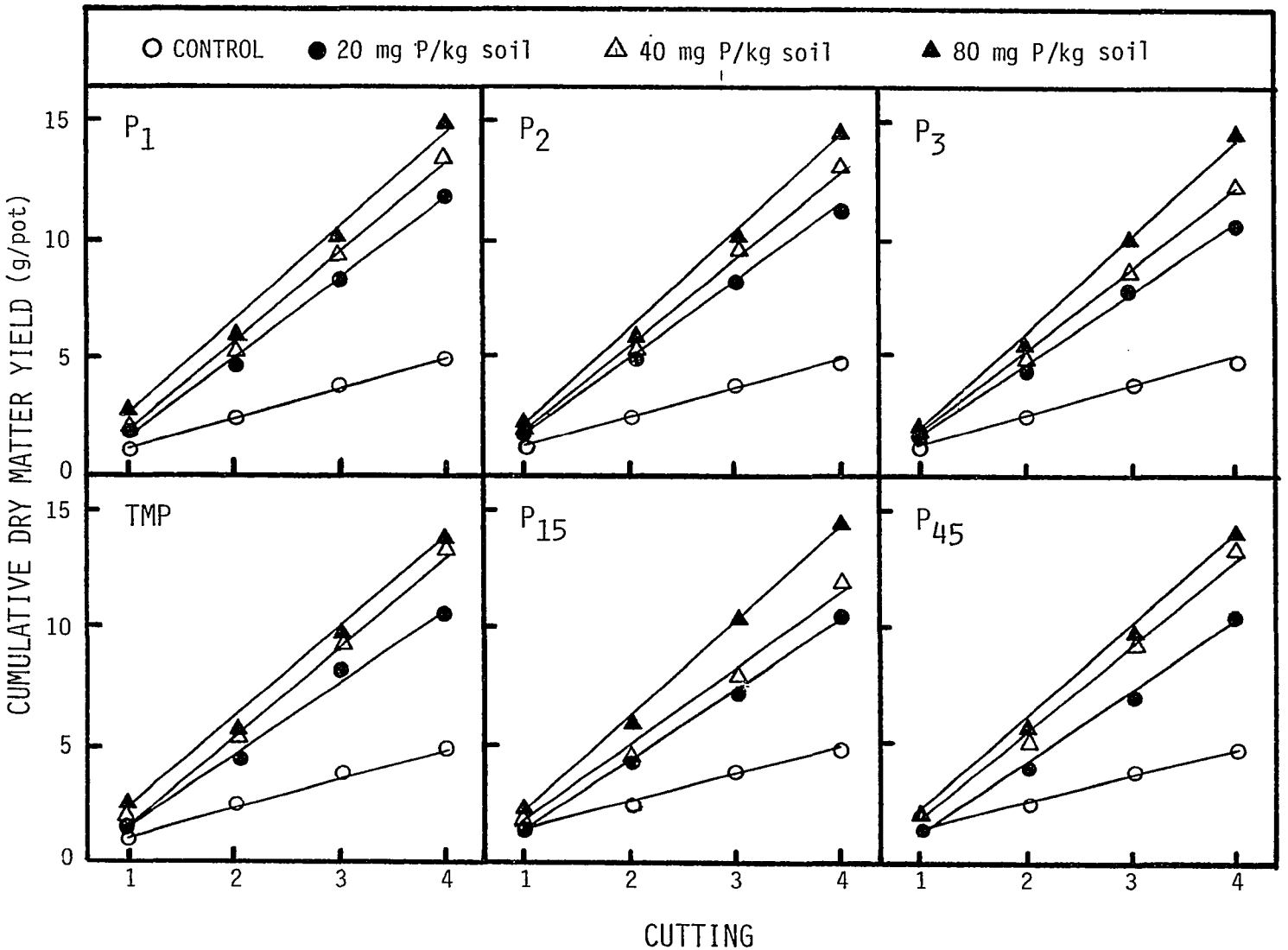

O CONTROL
● 20 mg P/kg soil
△ 40 mg P/kg soil
▲ 80 mg P/kg soil
P1
P2
P3
TMP
P15
P45
CUMULATIVE DRY MATTER YIELD (g/pot)
CUTTING
0
5
10
15
1
2
3
4

Figure 13. Comparison of cumulative dry matter yields of four cuttings of ryegrass produced on Ida soil treated with various sources of P applied at three rates

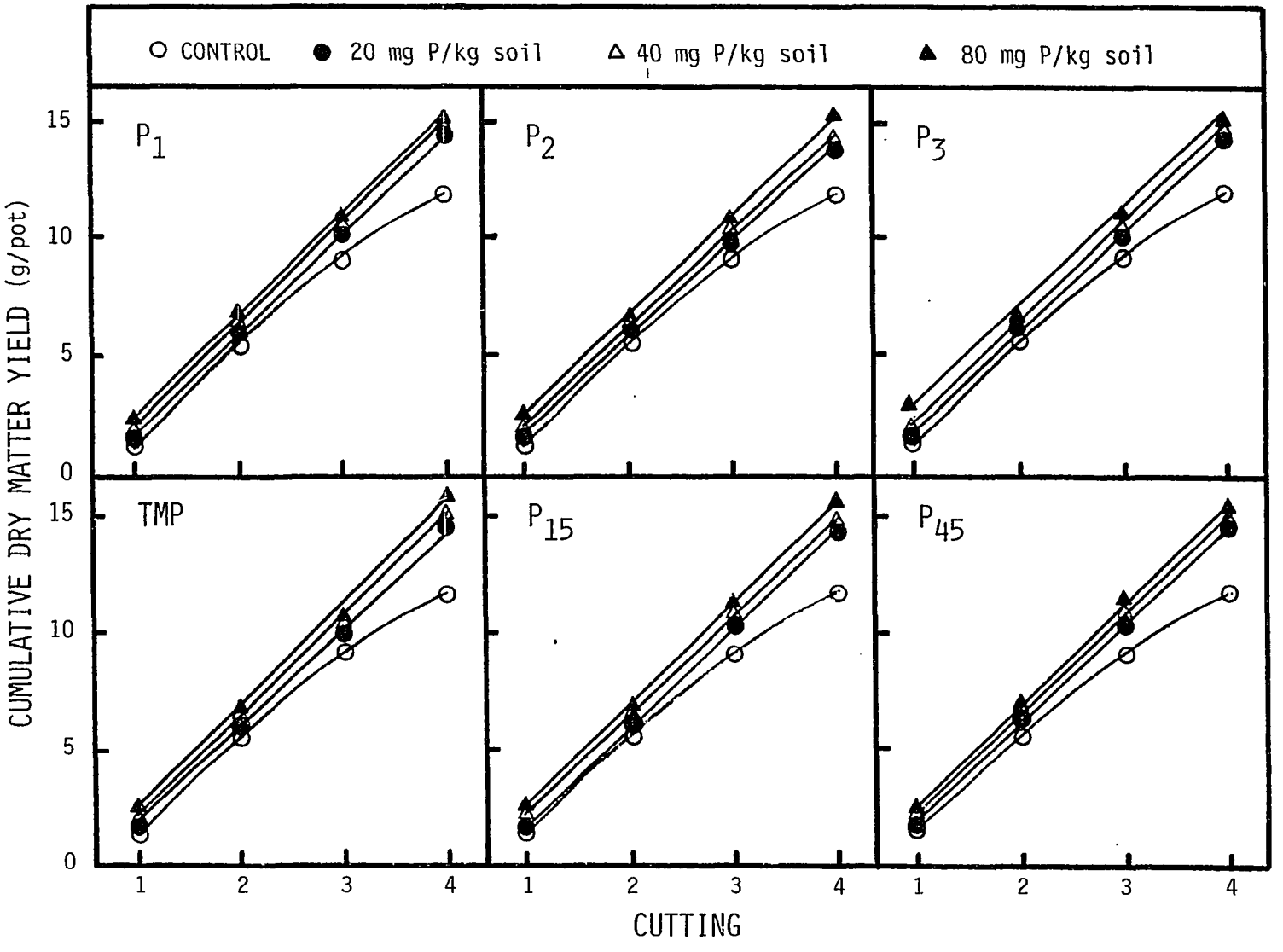

○ CONTROL
● 20 mg P/kg soil
△ 40 mg P/kg soil
▲ 80 mg P/kg soil
P1
P2
P3
TMP
P15
P45
CUMULATIVE DRY MATTER YIELD (g/pot)
0
5
10
15
1
2
3
4
CUTTING

Table 18. Analysis of variance of effects of soil, P compounds, and rates of P application on dry matter and P yields of ryegrass and corn

Source of variation	Mean squares					
	Rye grass			Corn		
	df[a]	Dry matter	P yield	df	Dry matter	P yield
Block	2			2		
Soils (S)	3	15.77***	39.01***	3	0.449***	1.91***
P compound (PC)	5	0.14	1.72	5	0.033**	0.40***
S x PC	15	0.20*	6.33*	15	0.014	0.07
Rate (R)	2	9.25***	1360.04***	2	0.885***	21.29***
R x S	6	2.02***	6.10	6	0.026*	0.36***
PC x R	10	0.14	1.64	10	0.013	0.06
PC x R x S	30	0.12	1.94	30	0.008	0.03
Error a	142	0.10	3.55	142[b]	0.010	0.06
Cut (C)	3	198.27***	15.32*			
C x S	9	6.52	46.33***			
PC x C	15	0.13	1.96			
PC x C x S	45	0.09	7.61***			
R x C	6	0.33**	7.72			
R x C x S	18	0.20*	3.83			
PC x R x C	30	0.08	2.30			
PC x R x C x S	90	0.10	3.56			
Error b	432	0.08	3.40			

[a]df - degrees of freedom.

[b]Degrees of freedom and associated mean squares are for the general error term.

*,**,***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

a dry matter response to increasing P additions. Analysis of variance showed that the main effect of soil and cuttings was highly significant but the main effect of P compounds was not significant. There was also a significant interactions of P compounds x soil. To further investigate this interaction, a Duncan's multiple range test was calculated (Table 19) to compare the effect of P sources on the total dry matter yields produced on each soil (controls were excluded because they had six replications, whereas all other treatments had only three replications). The dry matter yield of ryegrass produced on the calcareous Ida soil consistently showed no significant differences among P sources ($P < 0.05$), regardless of rate of P application. Although this trend did not occur in the other three acid soils, comparison of the dry matter yields produced with the various P sources showed that no P compound was superior over the others.

In the second study with corn on P-depleted soils, ANOVA of dry matter yields showed significant main effect of soil, P compound, and rate of application, but no significant interactions of these factors, except for rate x soil (Table 18). Comparison of the effect of P compounds on dry matter yield of corn are shown in Table 20. In general, the conclusions were similar to those of the ryegrass study in that no one P compound or group of P compounds is consistently superior to other P compounds in increasing the dry matter yield.

Table 19. Effect of sources and rates of P on dry-matter yield of four cuttings of ryegrass produced on four soils[a]

P compound	Dry matter yield at rate (mg P/kg soil) of P for soil specified					
	Clarion			Webster		
	20	40	80	20	40	80
	g/pot					
None[b]	12.5	12.5	12.5	12.4	12.4	12.4
P_1	15.2a	15.9a	15.7a	14.4ab	15.2b	15.8ab
P_2	15.4a	15.5a	16.0a	15.0a	15.2b	16.2a
P_3	14.5a	15.3a	15.9a	14.3ab	15.1b	14.5b
TMP	14.8a	15.2a	16.0a	14.6ab	15.8a	15.1ab
P_{15}	14.6a	15.3a	16.0a	14.0b	15.3ab	15.8ab
P_{45}	15.3a	15.6a	15.8a	14.8ab	15.2b	15.4ab
	Primghar			Ida		
	20	40	80	20	40	80
None	5.2	5.2	5.2	11.8	11.8	11.8
P_1	12.0a	13.8a	15.1a	14.5a	14.0a	14.3a
P_2	11.6ab	13.4a	14.8a	13.8a	13.7a	14.8a
P_3	10.9bc	12.9ab	14.9a	14.9a	13.8a	14.7a
TMP	11.3bc	13.5a	13.7a	13.6a	15.0a	15.3a
P_{15}	10.9c	12.2b	14.8a	14.7a	14.6a	15.1a
P_{45}	10.6c	13.6a	14.0a	14.3a	14.0a	14.8a

[a]Means followed by the same letter within any column are not significantly different at the 0.05 probability level using Duncan's multiple range test.

[b]None = control.

Table 20. Effect of sources and rates of P on dry matter yield of corn produced on four soils[a]

P compound	Dry matter yield at rate (mg P/kg soil) of P for soil specified					
	Clarion			Webster		
	20	40	80	20	40	80
	g/pot					
None[b]	1.08	1.08	1.08	1.19	1.19	1.19
P_1	1.21ab	1.25a	1.36a	1.22a	1.39ab	1.49b
P_2	1.07b	1.34a	1.40a	1.32a	1.46ab	1.55ab
P_3	1.14ab	1.33a	1.42a	1.34a	1.33b	1.47b
TMP	1.19ab	1.27a	1.44a	1.34a	1.58a	1.58a
P_{15}	1.24a	1.36a	1.41a	1.25a	1.36ab	1.47b
P_{45}	1.29a	1.36a	1.47a	1.39a	1.47ab	1.56ab

	Primghar			Ida		
	20	40	80	20	40	80
None	1.18	1.18	1.18	1.29	1.29	1.29
P_1	1.38a	1.37b	1.52b	1.48a	1.43a	1.50b
P_2	1.29a	1.59a	1.65ab	1.36ab	1.43a	1.50b
P_3	1.42a	1.60a	1.72ab	1.34b	1.41a	1.57ab
TMP	1.36a	1.56ab	1.75ab	1.39a	1.48a	1.55ab
P_{15}	1.31a	1.47ab	1.79a	1.39a	1.42a	1.57ab
P_{45}	1.43a	1.45ab	1.65ab	1.46a	1.47a	1.65a

[a]Means followed by the same letter within any column are not significantly different at the 0.05 probability level using Duncan's multiple range test.

[b]None = control.

Plant P Uptake

The percentages of P in the ryegrass tissue for the first cutting averaged across all P compounds were 0.28, 0.34, and 0.41% P for pots treated with 20, 40, and 80 mg P/kg soil, respectively. The percentage of P decreased with each cutting, resulting in the fourth cutting having corresponding averages for the ryegrass tissue of 0.17, 0.20, and 0.26% P. In the corn study, the percentage of P in the corn tissue was substantially lower than those of the fourth cutting of ryegrass, with an average across all P compounds of 0.13, 0.15, and 0.18% P for pots treated with 20, 40, and 80 mg P/kg soil (Appendix Table).

The amount of P recovered per pot in four cuttings of ryegrass is shown in Figure 14. There was a significant response to increasing rates of P application. For example, in the Clarion soil, the average P yield among P compounds per cutting increased from 1.41 to 6.04 mg P/kg soil for pots treated with 20 and 80 mg P/kg soil, respectively. A similar response occurred in the corn study (Fig. 15). The total amount of the applied P recovered by the four cuttings of ryegrass averaged over rates from all sources was 14.6 mg P/pot for Clarion soil, 15.9 mg P/pot for Webster soil, 19.1 mg P/pot for Primghar soil, and 15.7 mg P/pot for Ida soil, as calculated by the difference between treatment and control.

Figure 14. Effect of sources and rates of P on the amounts of P recovered by four cuttings of ryegrass produced on four soils; means of total P recovered followed by the same letter within each rate of P applied are not significantly different at the 0.05 probability level using the Duncan's multiple range test

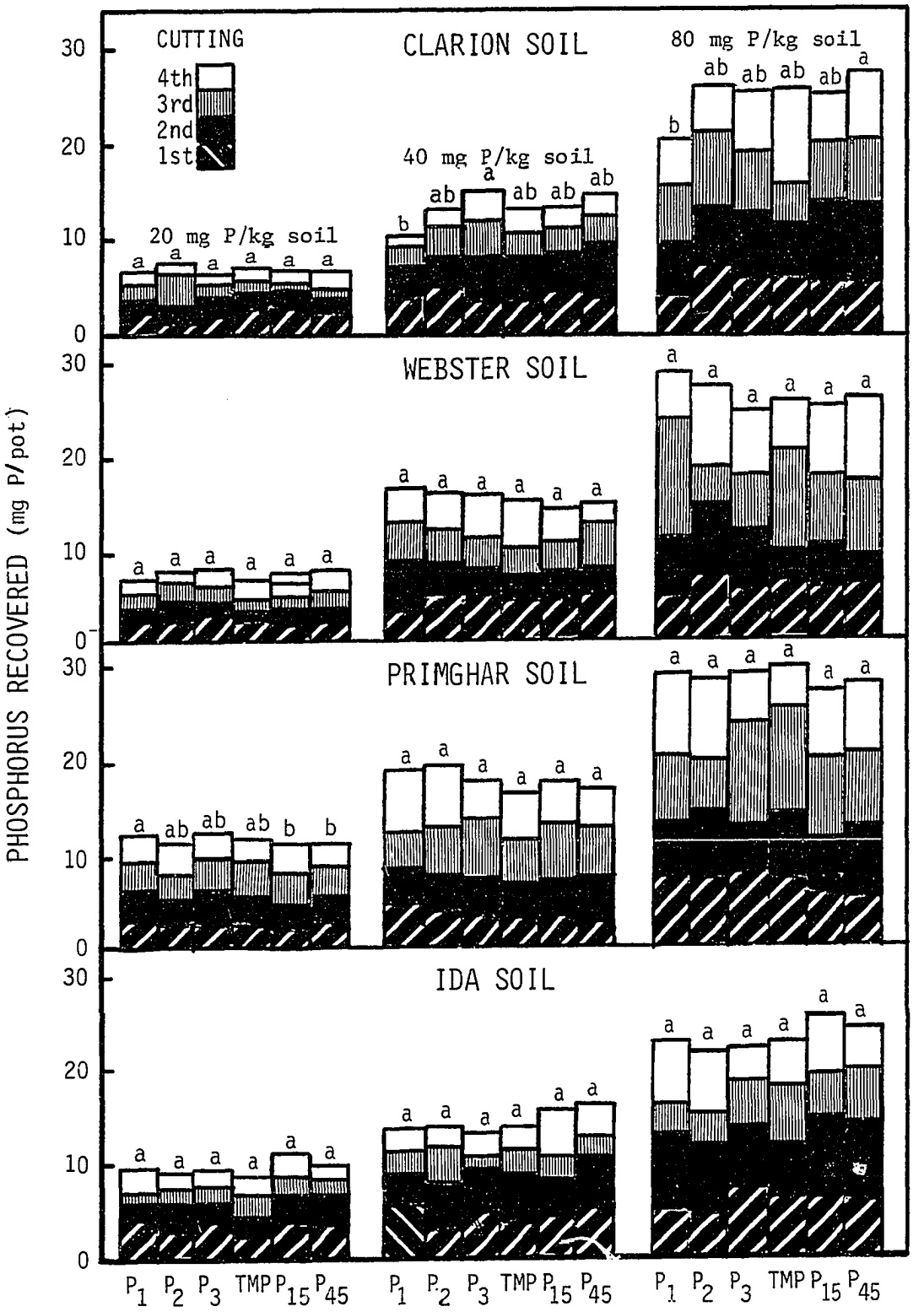
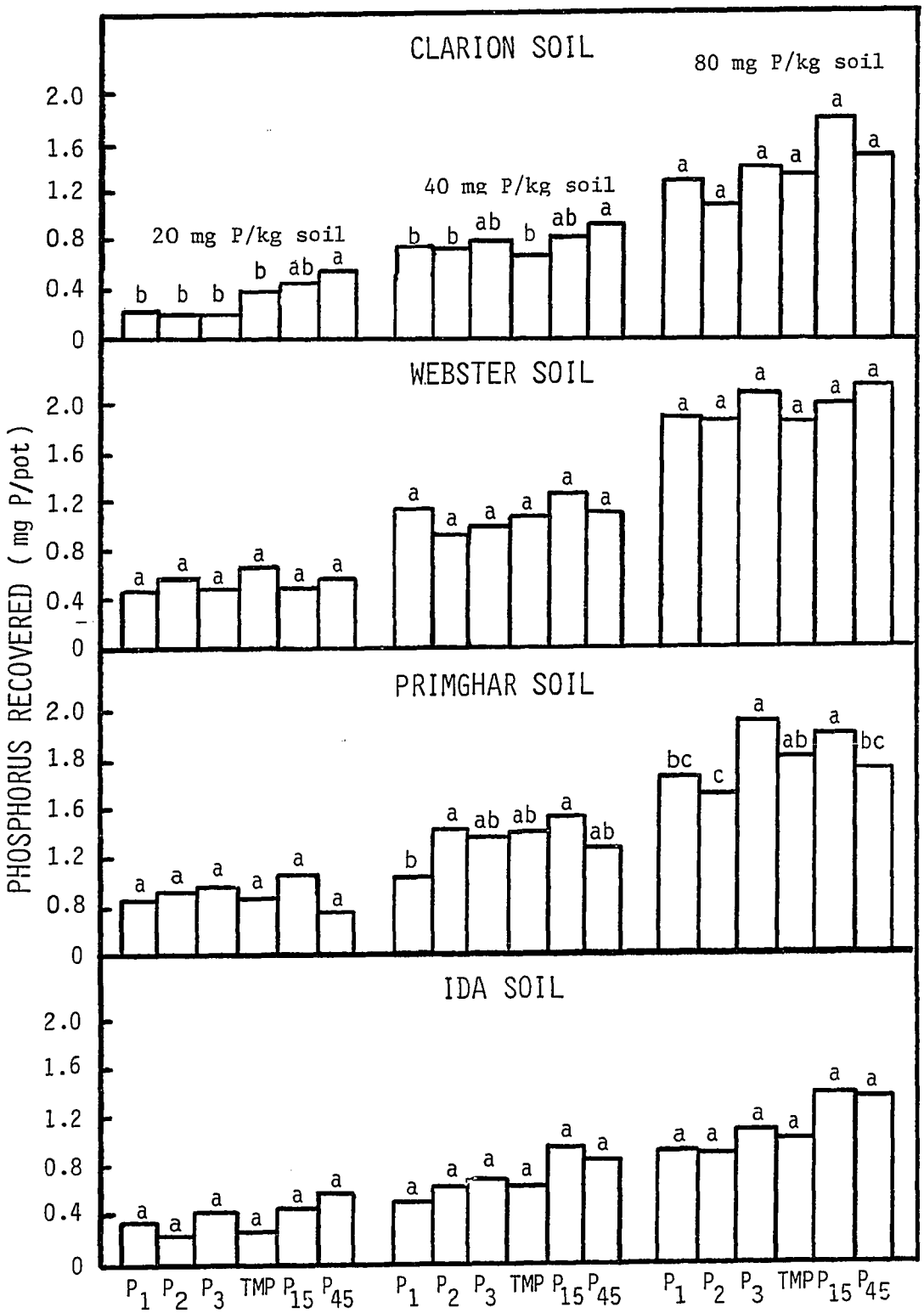

CUTTING
4th
3rd
2nd
1st
CLARION SOIL
WEBSTER SOIL
PRIMGHAR SOIL
IDA SOIL
20 mg P/kg soil
40 mg P/kg soil
80 mg P/kg soil
PHOSPHORUS RECOVERED (mg P/pot)
0
10
20
30
P1 P2 P3 TMP P15 P45

Figure 15. Effect of sources and rates of P on the amounts of P recovered in corn plants produced on four soils; means of total P recovered followed by the same letter within each rate of P applied are not significantly different at the 0.05 probability level using the Duncan's multiple range test


CLARION SOIL
80 mg P/kg soil
40 mg P/kg soil
20 mg P/kg soil
WEBSTER SOIL
PRIMGHAR SOIL
IDA SOIL
PHOSPHORUS RECOVERED (mg P/pot)
2.0
1.6
1.2
0.8
0.4
0
P1 P2 P3 TMP P15 P45

Analysis of variance showed that there was no significant main effect of P compounds in the ryegrass study, but there was significant soil x P compound interaction (Table 18). However, applying the Duncan's multiple range test for total P recovery (treatment minus control) showed very few differences among P compounds (Fig. 14). Furthermore, analysis of the data for each cutting indicated that the P availability from the various P sources were similar regardless of the cutting. The results with corn showed that the main effect of P compounds was significant but the interaction of soil x P compound was not significant (Table 18). As was the case with ryegrass, the results with corn again showed few significant differences between P compounds using the Duncan's multiple range test (Fig. 15). Figures 14 and 15 show that there is no one polyphosphate or group of polyphosphates that are superior to orthophosphate in terms of supplying P to plants.

The percentages of the added P recovered by four cuttings of ryegrass and one cropping of corn from each rate of application of the phosphates studied are shown in Tables 21 and 22, respectively. In general, the percentage of P recovered varied markedly among the four soils used. Although the P application rate affected the percentage of P recovered by plants, the trend was not consistent among the phosphate compounds and soils.

Considering all the P sources and rates of P application,

Table 21. Percentage recovery of P by four cuttings of ryegrass from P compounds added to soils

P compound	Rate of application	P recovered by ryegrass from soil specified[a]			
		Clarion	Webster	Primghar	Ida
	mg P/kg soil	----------%----------			
P_1	20	14.7	18.1	33.4	25.7
	40	13.9	28.3	25.1	18.9
	80	13.9	21.4	19.8	15.7
P_2	20	19.9	24.8	30.4	25.0
	40	18.1	21.4	25.3	18.1
	80	18.0	18.5	18.8	15.5
P_3	20	15.1	21.5	31.5	25.3
	40	25.2	21.3	23.9	18.3
	80	17.7	16.7	19.5	15.8
TMP	20	22.1	15.2	29.4	24.5
	40	19.2	19.9	22.2	19.3
	80	17.4	17.7	19.8	16.2
P_{15}	20	13.2	18.5	25.6	31.2
	40	18.8	19.4	23.6	21.9
	80	17.1	17.3	18.5	19.1
P_{45}	20	13.3	25.7	27.9	35.1
	40	21.5	22.2	22.5	18.9
	80	18.6	18.3	18.6	18.0

[a]Means of 3 replications.

Table 22. Percentage recovery of P by corn from P compounds added to soils

P compound	Rate of application	P recovered by corn from soil specified[a]			
		Clarion	Webster	Primghar	Ida
	mg P/kg soil	----------%----------			
P_1	20	7.3	15.2	15.3	13.6
	40	9.0	18.9	10.9	8.6
	80	10.8	16.0	11.8	7.8
P_2	20	7.2	18.0	16.7	8.1
	40	8.9	15.4	17.3	10.9
	80	9.4	14.2	10.7	7.8
P_3	20	6.8	14.0	17.3	14.6
	40	13.1	16.3	16.1	11.4
	80	10.6	17.1	15.8	9.1
TMP	20	8.7	21.7	15.5	8.3
	40	10.9	17.9	16.3	10.4
	80	10.9	14.2	13.6	8.5
P_{15}	20	15.6	15.6	20.9	15.7
	40	13.9	20.8	18.8	15.9
	80	15.1	16.4	14.9	11.6
P_{45}	20	11.4	16.7	10.7	20.3
	40	14.9	15.5	14.1	14.4
	80	12.4	17.4	12.0	11.4

[a]Means of 3 replications.

dry matter yield was significantly correlated (exponentially) with P uptake by ryegrass from all soils (Fig. 16). The correlation coefficients and the closeness of all points to the regression lines indicate that the effectiveness of a unit quantity of P taken up by corn or ryegrass in increasing the dry matter yield of these plants did not differ among the polyphosphates and the conventional orthophosphate fertilizer (Fig. 16 and Fig. 17). The results are consistent with other greenhouse studies where pyrophosphate and orthophosphate have been shown to be equally available to plants (Hughes and Hashimoto, 1971; Engelstad and Allen, 1971).

Extractable P

Immediately after completing each greenhouse experiment, soil samples were taken and analyzed for extractable P to assess the residual P effects from the various P compounds. The Primghar soil was clearly the most P-deficient soil. After the fourth cutting of ryegrass, the average amount of extractable P was 2.4 mg P/kg soil for all the P compounds at the application rate of 20 mg P/kg soil, which was approaching the amount of extractable P in the control (1.9 mg P/kg soil) (Table 23). Increasing rates of P application resulted in increased levels of extractable P for both experiments (Tables 23 and 24), regardless of P compound. Comparing the effect of P compounds on extractable P showed that none of the polyphos-

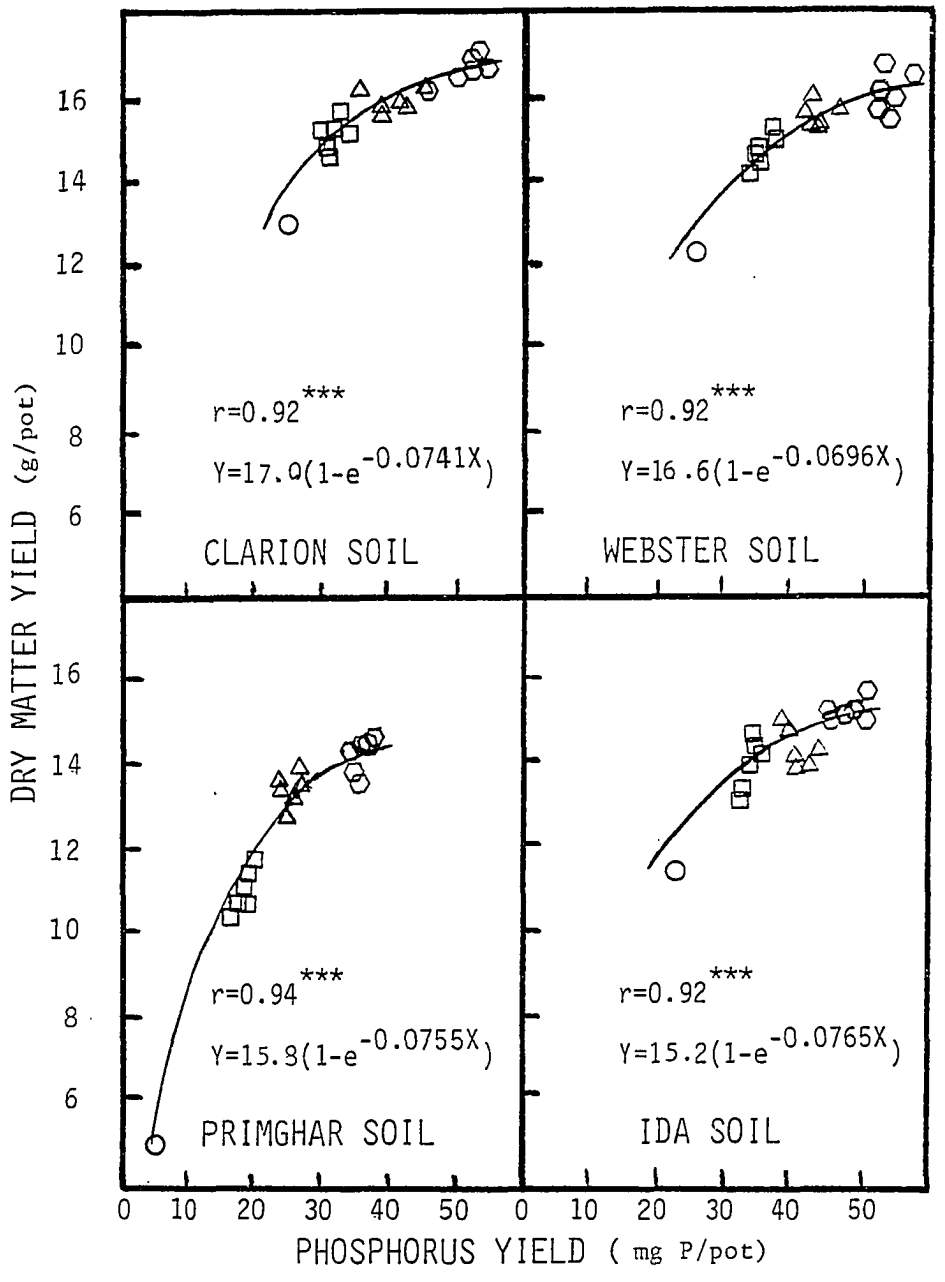


Figure 16. Relationship between total dry matter yields of four cuttings of ryegrass and P uptake from soils treated with P sources at three rates of P: ○ , control; □ , 20; △ , 40; and ⬡ , 80 mg P/kg soil

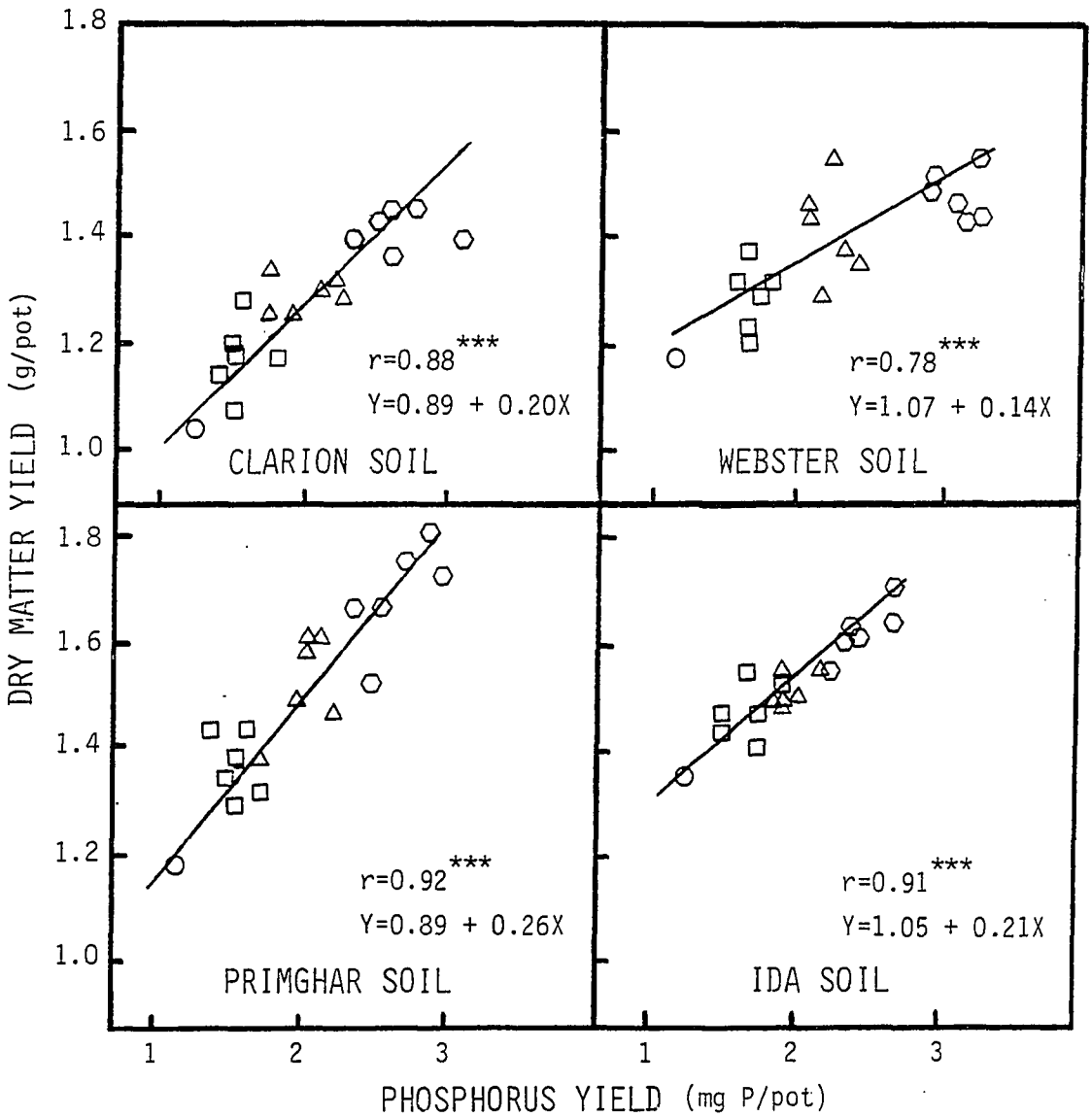


Figure 17. Relationship between total dry matter yields of corn and P uptake from soils treated with P sources at three rates of P: ○ , control; □ , 20; △ , 40; and ⬡ , 80 mg P/kg soil

Table 23. Effect of sources and rates of P on extractable P after four cuttings of ryegrass on four soils[a]

P compound	Extractable P in soil at applied P rate (mg P/kg) specified					
	Clarion[b]			Webster[b]		
	20	40	80	20	40	80
	mg P/kg soil					
None[c]	7.8	7.8	7.8	8.0	8.0	8.0
P_1	13.2a	16.6a	26.8a	9.0b	15.0a	22.9b
P_2	10.5b	15.2a	28.5a	9.8ab	13.5a	30.6a
P_3	11.1ab	12.2a	28.5a	10.0ab	13.4a	25.7ab
TMP	11.1ab	13.3a	31.4a	9.2ab	13.3a	27.2ab
P_{15}	10.8ab	16.0a	26.9a	10.1ab	13.0a	23.1b
P_{45}	9.4b	13.4a	27.9a	11.5a	13.7a	23.1b

	Primghar[b]			Ida[d]		
	20	40	80	20	40	80
None	1.9	1.9	1.9	7.2	7.2	7.2
P_1	2.6a	4.9a	12.9a	9.3ab	14.7a	26.1a
P_2	2.6a	4.0a	11.9a	9.1ab	17.1a	26.2a
P_3	2.4ab	4.7a	12.2a	7.8b	10.0a	25.7a
TMP	2.6ab	4.0a	11.6a	10.4a	12.5a	19.7a
P_{15}	2.0b	4.4a	9.9a	10.4a	13.2a	21.5a
P_{45}	2.5ab	3.8a	14.2a	9.3ab	14.0a	18.9a

[a]Means followed by the same letter within a column are not significantly different at the 0.05 probability level using Duncan's multiple range test.

[b]Extracted with Bray-Kurtz I reagent.

[c]None = control.

[d]Extracted with Olsen's reagent.

Table 24. Effect of sources and rates of P on extractable P after 35 days cropping with corn on four soils[a]

P compound	Extractable P in soil at applied P rate (mg P/kg) specified					
	Clarion[b]			Webster[b]		
	20	40	80	20	40	80
	mg P/kg soil					
None[c]	6.4	6.4	6.4	5.5	5.5	5.5
P_1	10.8a	19.7a	31.7a	10.6a	15.8a	23.7a
P_2	12.9a	17.0ab	29.2a	8.8ab	10.7b	20.0a
P_3	11.7a	14.5b	30.3a	8.0b	11.7b	19.5a
TMP	10.0a	17.7ab	29.8a	7.8b	11.2b	17.1a
P_{15}	12.6a	19.6a	36.1a	8.0b	12.2b	23.0a
P_{45}	10.4a	16.8ab	25.4a	7.2b	10.6b	17.6a
	Primghar[b]			Ida[d]		
	20	40	80	20	40	80
None	1.7	1.7	1.7	7.3	7.3	7.3
P_1	3.1ab	8.4a	19.3a	12.1a	21.3a	30.8a
P_2	3.8a	6.5b	16.5ab	12.3a	19.5a	35.5a
P_3	2.4b	5.2bc	12.8abc	9.9ab	21.2a	35.9a
TMP	2.2b	4.1c	10.4bc	8.8b	17.9a	37.5a
P_{15}	3.1ab	6.9ab	13.3abc	10.7ab	22.2a	43.9a
P_{45}	2.4b	4.7c	8.5c	10.5ab	13.8a	34.1a

[a]Means followed by the same letter within a column are not significantly different at the 0.05 probability level using Duncan's multiple range test.

[b]Extracted with Bray-Kurtz I reagent.

[c]None = control.

[d]Extracted with Olsen's reagent.

phates is consistently superior to orthophosphate in providing residual P after a short cropping period (35 days) with corn (Table 24) or after a long cropping period (120 days) with ryegrass (Table 23).

Results obtained in the study show that the rates of hydrolysis of linear oligomers in soils are rapid enough so as not to decrease, relative to orthophosphate, the P uptake by plants. Conversely, there appears to be no advantage in long-chain polyphosphates with regards to slow release effects, as is evident from similar dry matter and P yields, and residual P levels, regardless of chain length. Although TMP is not sorbed by soil minerals, the results suggest that it does not increase P availability over orthophosphate which is susceptible to sorption reactions. Polyphosphates containing high concentrations of P (up to 30% P) should be useful as high analysis P fertilizers. Similarly, industrial wastes containing linear oligomers (phosphate glass) should have potential as P sources for plants.

SUMMARY AND CONCLUSIONS

The objectives of this study were (1) to assess the hydrolysis of polyphosphates by corn roots, (2) to determine the degree of hydrolysis of polyphosphates added to soils, (3) to assess the factors affecting hydrolysis of polyphosphates added to soils, and (4) to evaluate the potential of polyphosphates as sources of P for plants.

The findings can be summarized as follows:

1. It was found that the optimal pH value for hydrolysis of P_3, P_5, and TMP by corn-root homogenate was 5.0, whereas for the hydrolysis of P_2, P_{15}, P_{25}, P_{35}, and P_{65}, it was 6.0. The rate of polyphosphate hydrolysis by corn-root homogenate was temperature dependent up to the point of enzyme inactivation (50°C).

2. Nonsterile intact corn roots showed higher rates of polyphosphate hydrolysis than sterile roots, especially with P_2, which indicated that rhizoplane microorganisms contribute to polyphosphate hydrolysis. The hydrolysis of all polyphosphates by sterile and nonsterile intact roots was very slow during the first 18 h at 30°C, but increased rapidly after 18 h with the oligomers $P \leq 25$. The oligomers P_{35} and P_{65} were quite resistant to hydrolysis by sterile and nonsterile roots after 48 h incubation at 30°C. An experiment with sterile intact roots in pyrophosphate solution suggested that pyrophosphatase was induced in corn roots in the presence of

its substrate. The order of hydrolysis rates of the oligomers by intact sterile corn roots was: $P_2 > P_3 > P_5 > TMP > P_{25} > P_{35} > P_{65}$.

3. Incubating seven linear oligomers ranging from P_2 to P_{65} and a cyclic polyphosphate (trimetaphosphate) for one week in four Iowa surface soils (500 ug P/g soil) showed that the amounts of orthophosphate (Pi) produced from the polyphosphates in air-dried soils ranged from 66 to 96% of those produced in field-moist soils. Increasing the temperature of incubation from 10 to 30°C increased the hydrolysis rates.

4. Incubation of polyphosphates with soils for 1 to 14 days indicated that, in general, the rate of hydrolysis decreased with increasing polyphosphate chain length. This was particularly evident during the first few days of incubation. In two acid soils (Webster and Muscatine), the rates of hydrolysis of the polyphosphates under waterlogged conditions were higher (5 to 16%) than those obtained for aerobic conditions. Calculation of the Q_{10} values suggested that, with the exception of the oligomers (P_{15} to P_{65}) in the three acid soils incubated at 10 to 20°C, the hydrolysis was dominated by enzyme reactions. Energy of activation (Ea) values for acid soils increased with increasing chain length, with a range from 10.7 to 71.2 kJ/mol. The relationship between the nonhydrolyzed polyphosphate and time of incubation showed that polyphosphate hydrolysis was controlled by two first-order

reactions. The initial faster rate (k_1) changed to a slower rate (k_2) at incubation times ranging from 2 to 7 days, depending on the oligomer, soil type, and soil moisture status. The k_1 and k_2 values obtained for soils incubated under aerobic conditions ranged from 1.7×10^{-4} to 3.3×10^{-5}/min and from 4.5×10^{-5} to 7.4×10^{-6}/min, respectively. The corresponding values for soils incubated under waterlogging conditions ranged from 2.4×10^{-4} to 2.6×10^{-5}/min and from 5.2×10^{-5} to 1.0×10^{-5}/min.

5. The results showed that from 27 to 47% of the total amount of the Pi produced in hydrolysis of polyphosphates in soil was due to chemical hydrolysis and the rest was due to biochemical reactions. Statistical analysis of the effect of 17 soil properties on the amounts of Pi produced from polyphosphates in 29 soils showed that, with the exception of P_2, the amounts of Pi produced from the individual polyphosphates were significantly correlated with soil pH, and negatively correlated with dithionite-extractable Al^{3+}. The amounts of Pi produced from certain polyphosphates were significantly correlated with water-soluble Ca^{2+}, pyrophosphatase activity (buffered and nonbuffered systems), organic C, specific surface area, CEC, total P, and inorganic P. The amounts of Pi produced from chemical hydrolysis of polyphosphates did not consistently show any significant simple correlations with any of the soil properties studied.

6. Multiple regression analysis showed that polyphos-

phate hydrolysis is affected by pH, water-soluble Ca^{2+}, and nonbuffered pyrophosphatase activity, all of which had significant positive partial regression coefficients. Soil properties that showed significant negative partial regression coefficients were percentage of clay (all polyphosphate compounds studied), water-soluble Mg^{2+} (P_2, P_3, TMP, P_5, P_{15}, and P_{25}), and dithionite-extractable Al^{3+} (P_2, P_3, TMP, P_{15}, P_{25}, P_{35}, and P_{65}). The R^2 values of these regression models ranged from 0.66*** to 0.88***. No such multiple regression model was found that adequately described the chemical hydrolysis of polyphosphates in soils.

7. In a greenhouse study, four linear polyphosphates (P_2, P_3, P_{15}, and P_{45}) and one cyclic polyphosphate (trimetaphosphate) were compared with orthophosphate (P_1), the conventional P source, for their ability to supply P to ryegrass and corn plants grown in four Iowa surface soils at the rates of 0, 20, 40, and 80 mg P/g soil. With all sources of P, dry matter yield and P yield increased with increasing rates of P application for both ryegrass and corn. Statistical analysis (Duncan's multiple range test) of P yields indicated that there were no P compounds that consistently showed significantly higher amounts of P recovery in either ryegrass or corn tissue. The percentages of the added P recovered by four cuttings of ryegrass and one cropping of corn from each rate of polyphosphate varied among the four soils. Although

the P application rate affected the percentage of P recovered by plants, the trend was not consistent among the polyphosphates. The percentages of P recovered ranged from 13 to 33% for four cuttings of ryegrass and from 7 to 21% for corn.

Considering all the P sources and rates of application, the dry matter yield was significantly correlated (exponentially) with P uptake by ryegrass from all four soils. The dry matter yield of corn was significantly correlated (linearly) with dry matter yield and P recovered in corn, indicating that the effectiveness of a unit amount of P taken up by plants in increasing the dry matter yield was similar among the polyphosphates and the conventional P fertilizer, orthophosphate. Measurements of the extractable P (Bray-Kurtz I on the three acid soils and Olsen's reagent on the alkaline soil) upon completion of each experiment, indicated that the residual P effects were similar among the P compounds after the short cropping period with corn (35 days) and the long cropping period with ryegrass (120 days).

8. Results obtained in the greenhouse study show that the rates of hydrolysis of linear oligomers in soils are rapid enough so as not to decrease, relative to orthophosphate, the P uptake by plants. Conversely, there appears to be no advantage in long-chain polyphosphate with regards to slow release effects, as is evident from similar dry matter and P yields, and residual P levels, regardless of chain

length. Although TMP is not sorbed by soil minerals, the results suggest that it does not increase P availability over orthophosphate. This is because TMP is hydrolyzed in soils to triphosphate, which in turn is hydrolyzed to pyrophosphate and orthophosphate, all of which are susceptible to sorption reactions. Polyphosphates containing high concentrations of P (up to 30%) should be useful as high analysis P fertilizers. Similarly, industrial wastes containing linear oligomers (phosphate glass) should have potential as P sources for plants.

LITERATURE CITED

Allmaras, R. D., and C. A. Black. 1962. Crop response to metaphosphate fertilizers. Soil Sci. Soc. Am. Proc. 26: 470-475.

Asanuma, S., H. Tanaka, and M. Yatazawa. 1978. Effects of soil microorganisms on the growth and on the nitrogen and phosphorus absorption by rice seedlings. Soil Sci. Plant Nutr. 24: 207-219.

Barber, D. A., and B. C. Loughman. 1967. The effect of microorganisms on the absorption of inorganic nutrients by intact plants. II. Uptake and utilization of phosphate by barley plants grown under sterile and non-sterile conditions. J. Exp. Bot. 18: 170-176.

Barber, D. A., and A. D. Rovira. 1975. Rhizosphere microorganisms and the absorption of phosphate by plants. Ann. Rep. ARC Letcombe Lab. 1974: 27-28.

Barr, A. J., J. H. Goodnight, J. P. Sall, and J. I. Helwig. 1976. A user's guide to SAS. SAS Institute, Inc., Raleigh, North Carolina.

Barrow, N. J., and T. C. Shaw. 1975. Effects of ionic strength and nature of the cation on desorption of phosphate from soil. J. Soil Sci. 30: 53-68.

Bieleski, R. L. 1971. Enzymes changes in plants following changes in their mineral nutrition. p. 143-153. In R. M. Samish (ed.) Recent advances in plant nutrition. Gordon and Breach Sci. Pub., New York.

Bieleski, R. L. 1974. Development of an externally-located alkaline phosphatase as a response to phosphorus deficiency. p. 165-170. In R. L. Bieleski, A. R. Ferguson, and M. M. Cresswell (eds.) Mechanisms of regulation of plant growth. Bull. 12. The Royal Society of New Zealand, Wellington.

Blanchar, R. W., and L. R. Hossner. 1969. Hydrolysis, sorption, and plant uptake of phosphorus from 32 Corn Belt soils treated with ortho, pyro, tripoly, and trimetaphosphate. Soil Sci. Soc. Am. Proc. 33: 622-652.

Bowen, G. D., and A. D. Rovira. 1966. Microbial factor in short term phosphate uptake studies with plant roots. Nature (London) 211: 665-666.

Bowen, G. D., and A. D. Rovira. 1969. The influence of micro-organisms on the growth and metabolism of plant roots. p. 170-201. In W. J. Whittington (ed.) Root growth. Butterworths, London.

Brady, N. C. 1974. The nature and properties of soils. Macmillan Publishing co., New York.

Bremner, J. M., and C. S. Mulvaney. 1982. Total nitrogen. In A. L. Page, R. H. Miller, and D. R. Keeney (eds.) Methods of soil analysis, Part 2. 2nd edition. Agronomy 9: 595-622. American Society of Agronomy, Madison, Wisconsin.

Bundy, L. G., and J. M. Bremner. 1972. A simple titrimetric method for determination of inorganic carbon in soils. Soil Sci. Soc. Am. Proc. 36: 273-276.

Busman, L. M. 1984. Behavior of polyphosphates in soils. Unpublished Ph.D. dissertation. Library, Iowa State University, Ames, Iowa.

Busman, L. M., and M. A. Tabatabai. 1985. Hydrolysis of trimetaphosphate in soils. Soil Sci. Soc. Am. J. (in press).

Chae, Y. M., and M. A. Tabatabai. 1981. Sulfolipid and phospholipid in soils and sewage sludges in Iowa. Soil Sci. Soc. Am. J. 45: 20-25.

Chang, C. W., and R. S. Bandurski. 1964. Exocellular enzymes of corn roots. Plant Physiol. 39: 60-64.

Chang, C., and G. J. Racz. 1977. Effects of temperature and phosphate concentration on rate of sodium pyrophosphate and sodium tripolyphosphate hydrolysis in soils. Can. J. Soil Sci. 57: 271-278.

Chapman, H. D. 1965. Cation-exchange capacity. In C. A. Black (ed.) Methods of soil analysis, Part 2. Agronomy 9: 891-901. American Society of Agronomy, Madison, Wisconsin.

Clark, F. E. 1949. Soil micro-organisms and plant roots. Adv. Agron. 1: 242-248.

Clarkson, D. T. 1974. Ion transport and cell structure in plants. McGraw-Hill, London.

Clesceri, N. L., and G. F. Lee. 1965. Hydrolysis of condensed phosphates. II. Sterile environment. Int. J. Air Water Pollut. 9: 743-751.

Corbridge, D.E.C. 1974. The structural chemistry of phosphorus. p. 127-171. Esevier Scientific Publ. Co., New York.

Cosgrove, D. J. 1967. Metabolism of organic phosphates in soil. p. 216-228. In A. D. McLaren and G. H. Peterson (eds.) Soil biochemistry. Vol. 1. Marcel Dekker, Inc., New York.

Czech, F. W., and T. P. Hrychyshyn. 1966. Some aspects of ion exchange chromatographic analysis of condensed phosphates. p. 163-166. In Automation in analytic chemistry. Technicon Symposia.

Dash, R. N., S. K. Mohanty, and S. Patnaik. 1979. Tracer studies on the relative efficiency of ammonium nitrate-phosphates and ammonium polyphosphates for rice on different soil types. Oryza 16: 47-56.

Dick, W. A. 1980. Phosphatases in the soil environment. Unpublished Ph.D. dissertation. Library, Iowa State University, Ames, Iowa.

Dick, W. A., and M. A. Tabatabai. 1977a. An alkaline oxidation method for determination of total phosphorus in soils. Soil Sci. Soc. Am. Proc. 41: 511-514.

Dick, W. A., and M. A. Tabatabai. 1977b. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. J. Environ. Qual. 6: 82-85.

Dick, W. A., and M. A. Tabatabai. 1978. Inorganic pyrophosphatase activity of soils. Soil Biol. Biochem. 10: 59-65.

Dick, W. A., and M. A. Tabatabai. 1984. Kinetic parameters of phosphatases in soils and organic waste materials. Soil Sci. 137: 7-15.

Dick, W. A., N. G. Juma, and M. A. Tabatabai. 1983. Effects of soils on acid phosphatase and inorganic pyrophosphatase of corn roots. Soil Sci. 136: 19-25.

Difco Laboratories. 1953. Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. Detroit, Michigan.

Drews, G. 1962. The cytochemistry of polyphosphates. Colloq. Intern. Center Natl. Tech. Sci. (Paris) 106: 533-539.

Engelstad, O. P., and S. E. Allen. 1971. Ammonium pyrophosphate and ammonium orthophosphate as phosphorus sources: Effects of soil temperature, placement, and incubation. Soil Sci. Soc. Am. Proc. 35: 1002-1004.

Engelstad, O. P., and G. L. Terman. 1980. Agronomic effectiveness of phosphate fertilizers. In F. E. Khasaweneh et al. (ed.) The role of phosphorus in agriculture. Agronomy 12: 316-317. American Society of Agronomy, Madison, Wisconsin.

Estermann, E. F., and A. D. McLaren. 1961. Contribution of rhizoplane organisms to the total capacity of plants to utilize organic nutrients. Plant Soil 15: 243-260.

Florkin, M., and E. H. Stotz. 1964. Comprehensive biochemistry. Vol. 13, 3rd edition. Elsevier North-Holland, Inc., New York.

Gahan, P. B., and A. J. Maple. 1966. The behavior of lysome-like particles during cell differentiation. J. Exp. Bot. 17: 161-166.

Gerretsen, F. C. 1948. The influence of micro-organisms on the phosphate intake by the plant. Plant Soil 1: 51-85.

Ghonsikar, C. P., and R. H. Miller. 1973. Soil inorganic polyphosphates of microbial origin. Plant Soil 38: 651-655.

Gilliam, J. W. 1970. Hydrolysis and uptake of pyrophosphate by plant roots. Soil Sci. Soc. Am. Proc. 34: 83-86.

Gilliam, J. W., and E. C. Sample. 1968. Hydrolysis of pyrophosphate in soils: pH and biological effects. Soil Sci. 106: 352-357.

Graham, T. 1833. Researches on the arsenites, phosphates, and modification of phosphoric acid. Phil. Trans. Roy. Soc. London 123: 253-284.

Greaves, M. P., and D. M. Welby. 1965. A study of breakdown of organic phosphates by microorganisms from root region of certain pasture grasses. J. Appl. Bacteriol. 28: 454-465.

Gunst, R. F., J. T. Webster, and R. L. Mason. 1976. A comparison of least squares and latent root regression estimators. Technometrics 18: 75-83.

Hall, J. L. 1969. Histochemical localization of β-glycerophosphatase activity in young root tips. Ann. Bot. 33: 399-406.

Hall, J. L., and V. S. Butt. 1968. Localization and kinetic properties and β-glycerophosphatase in barley roots. J. Exp. Bot. 19: 276-287.

Hall, J. L., and C.A.M. Davie. 1971. Localization of acid hydrolase activity in Zea mays L. root tips. Ann. Bot. 35: 849-855.

Halstead, R. L., and R. B. McKercher. 1975. Biochemistry and cycling of phosphorus. p. 31-63. In E. A. Paul and A. D. McLaren (eds.) Soil biochemistry. Vol. 4. Marcel Dekker Inc., New York.

Harold, F. M. 1963. Inorganic polyphosphates of high molecular weight from Aerobacter aerogenis. J. Bacteriol. 86: 885-887.

Harold, F. M. 1965. Regulatory mechanisms in the metabolism of inorganic polyphosphate in Aerobacter aerogenis. Colloq. Intern. Center Natl. Rech. Sci. (Paris) 124: 307-315.

Harold, F. M. 1966. Inorganic polyphosphates in biology: Structure, metabolism, and function. Bacteriol. Rev. 30: 772-794.

Hashimoto, I., and J. R. Lehr. 1973. Mobility of polyphosphates in soil. Soil Sci. Soc. Am. Proc. 37: 36-41.

Hashimoto, I., and Z. T. Wakefield. 1974. Hydrolysis of pyrophosphate in soils: Response to temperature and effect on heavy-metal uptake by plants. Soil Sci. 118: 90-94.

Hashimoto, I., J. D. Hughes, and O. D. Philen, Jr. 1969. Reaction of triammonium pyrophosphate with soils and soil minerals. Soil Sci. Soc. Am. Proc. 33: 401-405.

Heald, W. R. 1965. Calcium and magnesium. In C. A. Black (ed.) Methods of soil analysis, Part 2. Agronomy 9: 999-1010. American Society of Agronomy, Madison, Wisconsin.

Healy, R. M., and M. L. Kilpatrick. 1955. A kinetic study of the hydrolysis of trimetaphosphates. J. Am. Chem. Soc. 77: 5258-5264.

Henao, Julio. 1976. Soil variables for regression of Iowa corn yields on soil, management, and climatic variables. Unpublished Ph.D. dissertation. Library, Iowa State University, Ames, Iowa.

Hoffman-Ostenhof, and L. Slechta. 1957. Transferring enzymes in the metabolism of inorganic polyphosphates and pyrophosphates. Proc. Intern. Symp. Enzyme Chem., Tokyo, Kyoto 2: 180-189.

Hossner, L. R., and J. R. Melton. 1970. Pyrophosphate hydrolysis of ammonium, calcium, and calcium ammonium pyrophosphate in selected Texas soils. Soil Sci. Soc. Am. Proc. 34: 801-805.

Hossner, L. R., and D. P. Phillips. 1971. Pyrophosphate hydrolysis in flooded soil. Soil Sci. Soc. Am. Proc. 35: 379-383.

Hughes, J. D., and I. Hashimoto. 1971. Triammonium pyrophosphate as a source of phosphorus for plants. Soil Sci. Soc. Am. Proc. 35: 643-646.

Hughes, D. E., S. F. Conti, and R. C. Fuller. 1963. Inorganic polyphosphate metabolism in Chlorobium thiosulfatophilum. J. Bacteriol. 85: 577-584.

Jackson, M. L. 1956. Soil chemical analysis. Published by the author, Madison, Wisconsin.

Johnson, R. A., and D. W. Wichern. 1982. Applied multivariate statistical analysis. p. 313-315. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Juma, N. G. 1976. Phosphatase activity of soils and of corn and soybean roots. Unpublished M.S. thesis. Library, Iowa State University, Ames, Iowa.

Karl-Kroupa, E., C. F. Callis, and E. Seifter. 1957. Stability of condensed phosphates in very dilute solutions. Ind. Eng. Chem. 49: 2061-2062.

Kilmer, V. J., and L. T. Alexander. 1949. Methods of making mechanical analysis of soils. Soil Sci. 68: 15-24.

Kiss, S., M. Dragan-Bularda, and D. Radulescu. 1975. Biological significance of enzymes accumulated in soil. Adv. Agron. 27: 25-87.

Klein, C. R. 1981. Specific surface area and selected related properties of some Iowa soils. M.S. thesis. Library, Iowa State University, Ames, Iowa.

Knudsen, D. 1980. Recommended phosphorus tests. p. 14-16. In W. C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. Bull. No. 499. North Dakota Agr. Exp. Stn., Fargo, North Dakota.

Kulaev, I. S. 1979. The biochemistry of inorganic polyphosphates. John Wiley & Sons, New York.

Lawry, N. H., and T. E. Jensen. 1979. Deposition of condensed phosphate as an effect of varying sulfur deficiency in Cyanobacterium Synechococcus sp. (Anacystis nidulans). Arch. Microbiol. 120: 1-7.

Lindsay, W. L., A. W. Frazier, and H. F. Stephenson. 1962. Identification of reaction products from phosphate fertilizers in soils. Soil Sci. Soc. Am. Proc. 26: 446-452.

Lynch, J. M. 1976. Products of soil microorganisms in relation to plant growth. CRC Critical Rev. Microbiol. 5: 67-107.

Lyons, J. W. 1964. Sodium tri(poly)phosphate in the kaolinite-water system. J. Colloid Sci. 19: 399-412.

Martin, J. B., and D. M. Doty. 1949. Determination of inorganic phosphate. Anal. Chem. 21: 965-967.

McGeorge, W. T. 1939. Factors influencing the availability of native soil phosphate and phosphate fertilizers in Arizona soils. Arizona Agr. Exp. Stn. Tech. Bull. 82.

McLean, J., and P. B. Gahan. 1970. The distribution of acid phosphatases and esterases in differentiating roots of Vicia faba. Histochem. 24: 41-49.

Mebius, L. J. 1960. A rapid method for the determination of organic carbon in soils. Anal. Chim. Acta 22: 120-124.

Mosse, B. 1975. A microbiologist's view of root anatomy. p. 39-66. In N. Walker (ed.) Soil microbiology. Butterworths, London.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27: 31-36.

Nelson, D. W., and L. E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65: 109-112.

Ohashi, S., and J. R. Van Wazer. 1964. Paper chromatography of very long chain polyphosphates. Anal. Chem. 35: 1984-1985.

Olsen, S. R., and L. A. Dean. 1965. Phosphorus. In C. A. Black (ed.) Methods of soil analysis, Part 2. Agronomy 9: 1035-1049. American Society of Agronomy, Madison, Wisconsin.

Ozanne, P. G. 1980. Phosphate nutrition of plants--a general treatise. p. 559-589. In F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (eds.) The role of phosphorus in agriculture. American Society of Agronomy, Madison, Wisconsin.

Patrick, W. H., Jr., and I. C. Mahapatra. 1968. Transformation and availability to rice of nitrogen and phosphorus in waterlogged soils. Adv. Agron. 20: 323-359.

Patrick, W. H., and W. J. Fontenot. 1976. Growth and mineral composition of rice at various moisture tensions and oxygen levels. Agron. J. 68: 325-329.

Pepper, I. L., R. H. Miller, and C. P. Ghonsikar. 1976. Microbial inorganic polyphosphates: Factors influencing their accumulation in soil. Soil Sci. Soc. Am. J. 40: 872-875.

Philen, O. D., Jr., and J. R. Lehr. 1967. Reactions of ammonium polyphosphates with soil minerals. Soil Sci. Soc. Am. Proc. 31: 196-199.

Ponnamperuma, F. N. 1965. Dynamic aspects of flooded soils and the nutrition of the rice plant. In The mineral nutrition of the rice plant. Proc. Intern. Rice Res. Inst., John Hopkins Press, Baltimore, Maryland.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24: 29-96.

Pratt, P. F. 1965. Potassium. In C. A. Black (ed.) Methods of soil analysis, Part 2. Agronomy 9: 1022-1030. American Society of Agronomy, Madison, Wisconsin.

Ramirez-Martinez, J. R. 1968. Organic phosphorus mineralization and phosphatase activity in soils. Folia Microbiol. 13: 161-174.

Ratner, E. I., and S. A. Samoilova. 1955. The exocellular phosphatase activity of roots. Ficiol. Rast. 2: 30-40.

Reid, M. S., and R. L. Bieleski. 1970. Changes in phosphatase activity in phosphorus-deficient Spirodela. Planta 94: 273-281.

Ridge, E. H., and A. D. Rovira. 1971. Phosphatase activity of intact young wheat roots under sterile and nonsterile conditions. New Phytol. 70: 1017-1026.

Roche, J. 1950. Phosphatase. In J. B. Sumner and K. Myrback (eds.) The enzymes. Vol. 1, Part 1. Academic Press, New York.

Rogers, H. T. 1942. Dephosphorylation of organic phosphorus compounds by soil catalysts. Soil Sci. 54: 439-446.

Rogers, H. T., R. W. Pearson, and W. H. Pierre. 1940. Absorption of organic phosphorus by corn and tomato plants and the mineralizing action of exoenzyme systems of growing roots. Soil Sci. Soc. Am. Proc. 5: 285-291.

Rolston, D. E., R. S. Rauschkolb, and D. L. Hoffman. 1975. Infiltration of organic phosphate compounds in soil. Soil Sci. Soc. Am. Proc. 39: 1089-1094.

Rovira, A. D., and B. M. McDougall. 1967. Microbiological and biochemical aspects of the rhizosphere. p. 418-463. In A. D. McLaren and G. H. Peterson (eds.) Soil biochemistry. Edward Arnold, London.

Savant, N. K., and G. J. Racz. 1972. Hydrolysis of sodium pyrophosphate and tripolyphosphate by plant roots. Soil Sci. 113: 18-22.

Schmidt, G., and M. Laskowski. 1961. Phosphate ester cleavage (survey). p. 3-35. In P. D. Boyer, H. Lardy, and K. Myrback (eds.) The enzymes. 2nd edition. Academic Press, New York.

Searle, P.G.E., and J. D. Hughes. 1977. Cation activation of pyrophosphatase from soil microorganisms. Soil Biol. Biochem. 9: 153-156.

Shaykh, M. M., and L. W. Roberts. 1974. A histochemical study of phosphatases in root apical meristems. Ann. Bot. 38: 165-174.

Shen, C. Y., and F. W. Morgan. 1973. Hydrolysis of phosphorus compounds. p. 241-263. In E. J. Griffith et al. (eds.) Environmental phosphorus handbook. John Wiley & Sons, New York.

Skujins, J. J. 1967. Enzymes in soil. p. 371-414. In A. D. McLaren and G. H. Peterson (eds.) Soil biochemistry. Vol. 1. Marcel Dekker, New York.

Skujins, J. 1976. Extracellular enzymes in soil. Critical Reviews in Microbiology 4: 383-421.

Skujins, J., J. L. Braal, and A. D. McLaren. 1962. Characterization of phosphatase in a terrestrial soil sterilized with an electron beam. Enzymologia 25: 125-133.

Sommers, L. E., and D. W. Nelson. 1972. Determination of total phosphorus in soils: A rapid perchloric acid digestion procedure. Soil Sci. Soc. Am. Proc. 36: 902-904.

Spencer, D. 1954. The effect of molybdate on the activity of tomato acid phosphatases. Aust. J. Biol. Sci. 7: 151-160.

Spier, T. W., and D. J. Ross. 1978. Soil phosphatase and sulphatase. p. 197-250. In R. G. Burns (ed.) Soil enzymes. Academic Press, New York.

Starkey, R. L. 1958. Interrelations between microorganisms and plant roots in the rhizosphere. Bacteriol. Rev. 22: 154-172.

Steel, G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York.

Subbarao, Y. V., R. Ellis, Jr., G. M. Paulsen, and J. V. Paukstellis. 1977. Kinetics of pyro- and tripolyphosphate hydrolysis in the presence of corn and soybean roots as determined by NMR spectroscopy. Soil Sci. Soc. Am. J. 41: 316-318.

Sutton, C. D., and S. Larsen. 1964. Pyrophosphate as a source of phosphorus for plants. Soil Sci. 97: 196-201.

Sutton, C. D., D. Gurnary, and S. Larsen. 1966. Pyrophosphate as a source of phosphorus for plants. II. Hydrolysis and initial uptake by a barley crop. Soil Sci. 101: 199-294.

Szymona, M. 1962. Purification and properties of the new hexokinase utilizing inorganic polyphosphate. Acta Biochem. Polon. 9: 165-180.

Szymona, M., and W. Ostrowski. 1964. Inorganic polyphosphate glucokinase of Mycobacterium phlei. Biochem. Biophys. Acta 85: 283-295.

Tabatabai, M. A. 1982. Soil enzymes. p. 903-943. In A. L. Page, R. H. Miller, and D. R. Kenney (eds.) Methods of soil analysis, Part 2: Chemical and microbiological properties. American Society of Agronomy, Madison, Wisconsin.

Tabatabai, M. A., and W. A. Dick. 1979. Distribution and stability of pyrophosphatase in soils. Soil Biol. Biochem. 11: 655-659.

Takai, Y., T. Koyama, and T. Kamura. 1956. Microbial metabolism in reduction of paddy soils. Part 1. Soil Plant Food 2: 63-66.

Terman, G. L. 1975. Agronomic results with polyphosphate fertilizers. Phosph. Agr. 65: 21-26.

Terman, G. L., and L. F. Seatz. 1956. Crop response to metaphosphate fertilizers. Soil Sci. Soc. Am. Proc. 20: 375-378.

Thilo, E. 1962. Condensed phosphates and arsenates. Adv. Inorg. Chem. Radiochem. 4: 1-77.

Thilo, E. 1965. The structural chemistry of condensed inorganic phosphates. Angew. Chem., International Edition 4: 1061-1071.

Tinker, P. B. 1980. Role of rhizosphere microorganisms in phosphorus uptake by plants. In E. F. Khasawneh, E. C. Sample, and E. J. Kamprath (eds.) The role of phosphorus in agriculture. American Society of Agronomy, Madison, Wisconsin.

Tisdale, S. L., and E. Winters. 1953. Crop response to calcium metaphosphate on alkaline soils. Agron. J. 45: 228-234.

Tsuge, T., and T. Yoshida. 1958. Polyphosphates. I. Evaluation of the availability of phosphorus in polyphosphates by plant test. Nippon Dojo Hiryogaku Zasshi 28: 497-502.

Van Wazer, J. R. 1958. Phosphorus and its compounds. Vol. I. Chemistry. Interscience Publishers, Inc., New York.

Vanghan, D., B. G. Ord, and R. E. Malcom. 1978. Effect of soil organic matter on some root surface enzymes and uptake into winter wheat. J. Exp. Bot. 29: 1337-1344.

Weil-Malherbe, H., and R. H. Green. 1951. The catalytic effect of molybdate on the hydrolysis of organic phosphate bonds. Biochem. J. 49: 386-392.

Winder, F. G., and J. M. Denny. 1957. The metabolism of inorganic polyphosphate in mycobacteria. J. Gen. Microbiol. 17: 573-585.

Wirda, A. 1959. Metachromatic granules of microorganisms. J. Bacteriol. 78: 664-670.

Young, R. D., and C. H. Davis. 1980. Phosphate fertilizers and process technology. p. 195-226. In F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (eds.) The role of phosphorus in agriculture. American Society of Agronomy, Madison, Wisconsin.

Zeffren, E., and P. L. Hall. 1973. The study of enzyme mechanisms. John Wiley & Sons, New York.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to:

Dr. M. A. Tabatabai, who as the Major Professor provided a keen interest in the work, encouragement, and time throughout this research project, and for his financial assistance;

Drs. I. C. Anderson, T. E. Fenton, D. J. Nevins, C. R. Stewart, and J. R. Webb for serving on the advisory committee;

Mrs. Ina Couture, for typing the manuscript;

His wife, Linda, for giving of her encouragement and sacrifices while this work was being done;

His parents Mr. and Mrs. Ben P. Dick who over many years have provided guidance and support which has been important to the author for the successful completion of the graduate study.

APPENDIX

Table 25. Properties of soils used in Part III

Soil	pH	Organic C	Total N	Phosphorus[a] O	Phosphorus[a] I	Extractable[b] Fe	Extractable[b] Al
		-------%------		-µg/g soil-		-mg/g soil--	
Storden	8.0	0.36	0.043	292	424	5.59	0.326
Hagener	6.0	0.92	0.093	499	84	4.71	0.545
Lester 2	6.8	1.26	0.150	151	174	3.74	0.294
Clarion	6.4	1.43	0.155	233	210	5.82	0.898
Ida	7.8	1.57	0.147	610	535	5.97	0.626
Ackmore	6.3	1.62	0.138	227	260	4.64	0.412
Hayden	6.5	1.68	0.201	218	122	3.90	0.471
Fayette 2	5.2	1.83	0.168	297	269	3.19	0.364
Tama 1	5.6	1.83	0.168	297	269	6.26	0.888
Downs	5.6	1.86	0.182	319	301	2.99	0.406
Edina	6.0	1.95	0.178	551	34	3.04	0.385
Grundy	6.5	1.99	0.191	626	62	3.52	0.497
Nicollet	7.0	2.00	0.183	456	197	4.29	0.754
Tama 2	7.5	2.04	0.197	178	497	6.23	0.604
Muscatine 3	6.7	2.05	0.220	322	184	5.39	0.738
Marshall	5.8	2.06	0.196	763	334	6.75	0.797
Shelby	6.4	2.46	0.231	569	47	8.04	1.208
Sharpsburg	5.7	2.54	0.229	561	214	6.17	1.005
Muscatine 2	7.3	2.60	0.233	358	295	4.31	0.487
Muscatine 1	5.8	2.63	0.227	330	285	4.16	0.722
Ames	6.7	2.99	0.223	192	202	2.39	0.225
Fayette 1	7.5	3.08	0.289	288	473	6.03	0.658
Webster	6.2	3.32	0.337	368	114	2.39	0.332
Lester 1	6.5	3.78	0.400	402	140	3.32	0.439
Harps	7.7	3.85	0.401	319	415	0.86	0.187
Canisteo 2	7.8	4.11	0.352	846	344	1.89	0.262
Canisteo 1	7.5	4.66	0.464	609	689	1.38	0.203
Okoboji	7.1	5.59	0.523	249	827	1.28	0.241
Chelsea	7.2	0.64	0.057	297	89	1.69	0.209

[a]O, organic P; I, inorganic P.

[b]Sodium dithionite-extractable Fe and Al.

[c]Water-extractable Ca and Mg.

[d]CEC, cation exchange capacity; ceq/kg soil.

[e]SA, surface area.

[f]BPA, buffered (pH 8.0) and NPA, nonbuffered pyrophosphatase activity expressed as µg Pi released/g soil/5 h.

Extractable[c]								
Ca	Mg	CEC[d]	$CaCO_3$	Clay	Sand	SA[e]	BPA[f]	NPA[f]
-µg/g soil--			---------%--------			m^2/g		
34.6	49.1	5.6	8.12	20	41	131	63	100
19.3	26.1	9.6	0	13	64	42	59	192
17.7	27.0	13.1	0	17	19	79	188	248
15.7	23.1	16.3	0	27	62	127	247	306
49.0	31.2	17.7	1.00	27	3	185	186	92
26.1	36.4	18.2	0	22	10	145	349	304
17.5	22.4	13.8	0	16	34	52	403	581
45.2	48.9	19.0	0	22	10	120	233	197
38.1	57.0	25.6	0	28	3	120	496	583
38.2	43.4	16.8	0	19	3	97	240	264
17.3	23.8	21.2	0	25	1	119	538	447
32.8	36.7	22.0	0	25	4	142	286	307
20.8	35.0	18.8	0	22	44	91	54	84
30.1	24.6	24.7	0.06	29	3	180	111	77
21.5	30.6	28.4	0	33	10	200	628	471
27.5	24.4	16.4	0	25	1	138	369	286
30.1	34.7	23.2	0	26	33	157	370	455
21.3	33.5	24.8	0	33	2	143	628	471
27.9	29.1	29.5	0.06	30	3	143	191	158
27.7	42.8	25.2	0	28	3	111	317	470
24.4	35.1	12.9	0	10	57	54	159	256
48.5	34.9	26.2	0.24	26	3	170	152	131
22.2	38.0	29.9	0	30	41	206	436	396
23.9	44.9	30.7	0	30	33	114	910	841
62.5	51.0	30.7	8.12	30	36	217	156	105
71.3	49.8	37.9	7.07	37	6	271	43	122
71.5	46.4	34.3	1.39	32	23	238	234	100
35.2	52.2	44.5	0.02	36	15	283	281	361
15.7	14.4	7.8	0	4	93	15	141	95

Table 26. The amounts of Pi produced from polyphosphates added to the soils of Table 10 and incubated for 7 days at 25°C

Soil	Amount of Pi produced for P compound specified							
	P2	P3	TMP	P5	P15	P25	P35	P65
	mg Pi/kg soil							
Muscatine 1	174	144	126	105	94	89	80	90
Muscatine 3	179	178	168	176	163	140	137	122
Tama 2	179	155	126	145	130	108	96	95
Lester 1	221	201	187	195	156	142	157	139
Lester 2	237	259	217	251	240	217	212	169
Fayette 2	137	176	120	199	144	166	164	120
Clarion	196	177	150	169	140	122	120	114
Hayden	286	301	251	265	254	244	205	231
Webster	204	195	177	171	136	124	115	105
Nicollet	84	114	105	127	120	117	114	90
Chelsea	310	323	288	265	232	229	224	228
Hagener	335	285	169	212	194	151	138	86
Edina	217	155	163	183	157	118	127	117
Sharpsburg	142	137	102	111	102	90	86	81
Ackmore	218	212	193	183	212	184	163	190
Ames	326	359	330	369	310	277	281	279
Marshall	174	138	114	130	120	110	101	81
Downs	194	143	125	112	173	171	157	102
Shelby	188	175	153	138	124	136	101	83
Grundy	168	172	148	160	148	141	145	148
Storden	161	193	232	231	218	222	239	190
Muscatine 2	155	179	168	181	181	173	158	165
Tama 1	183	185	192	216	200	186	182	164
Canisteo 1	259	303	270	255	202	270	243	235
Canisteo 2	262	270	254	266	264	274	270	256
Okoboji	178	187	176	184	193	158	142	152
Fayette 1	221	250	258	197	250	234	221	147
Harps	235	281	257	273	273	270	248	209
Ida	300	276	282	256	270	263	287	200

Table 27. The amounts of Pi produced from polyphosphates added to the steam sterilized soils of Table 10 and incubated for 7 days at 25°C

Soil	Amount of Pi produced for P compound specified							
	P2	P3	TMP	P5	P15	P25	P35	P65
	mg Pi/kg soil							
Muscatine	37	108	76	80	80	57	57	58
Muscatine	13	91	56	72	50	97	75	34
Tama	43	109	78	72	47	82	63	27
Lester	100	107	122	115	92	95	86	63
Lester	2	73	61	79	45	41	79	74
Fayette	106	101	95	58	72	96	51	55
Clarion	14	46	60	39	13	34	46	33
Hayden	15	87	29	92	76	64	79	66
Webster	110	110	123	107	114	38	52	17
Nicollet	52	106	61	120	112	84	85	70
Chelsea	106	73	147	104	39	48	43	53
Hagener	15	91	22	72	49	44	49	61
Edina	37	130	64	107	59	76	69	52
Sharpsburg	36	88	51	49	50	58	45	44
Ackmore	47	84	122	111	69	66	49	72
Ames	20	62	40	88	85	78	70	56
Marshall	108	29	92	105	110	53	65	73
Downs	186	131	96	87	96	43	52	47
Shelby	88	95	72	58	77	33	56	53
Grundy	27	93	76	120	108	56	41	70
Storden	26	38	111	62	30	60	42	92
Muscatine	137	140	124	86	96	96	93	80
Tama	48	113	108	107	101	87	86	55
Canisteo	75	129	55	63	79	20	41	121
Canisteo	68	166	210	189	199	160	186	150
Okoboji	34	47	103	121	98	128	27	33
Fayette	39	121	60	52	81	78	76	60
Harps	92	87	135	106	109	93	57	130
Ida	3	84	52	227	221	200	170	180

Table 28. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; first cutting, Clarion soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	2.35	4.02	0.303	7.41
1		0	2.79	4.38	0.235	5.60
1	P1	20	2.71	3.57	0.357	6.22
1	P1	40	2.53	4.69	0.444	6.71
1	P1	80	2.90	4.36	0.369	4.94
1	P2	20	2.79	4.30	0.289	5.82
1	P2	40	2.62	4.33	0.360	5.81
1	P2	80	2.79	4.18	0.470	5.90
1	P3	20	2.69	4.32	0.297	5.33
1	P3	40	2.90	4.14	0.297	5.09
1	P3	80	2.89	3.81	0.400	5.83
1	TMP	20	2.46	4.45	0.370	5.68
1	TMP	40	2.86	4.16	0.330	5.64
1	TMP	80	2.71	4.34	0.470	6.66
1	P15	20	2.59	4.36	0.323	6.34
1	P15	40	2.64	4.36	0.430	6.39
1	P15	80	2.79	4.36	0.410	5.61
1	P45	20	2.56	4.44	0.300	5.56
1	P45	40	2.75	4.51	0.421	6.23
1	P45	80	2.81	4.09	0.442	6.16
2		0	2.35	4.90	0.214	5.79
2		0	2.47	4.25	0.233	6.34
2	P1	20	2.41	5.23	0.371	6.47
2	P1	40	2.59	4.58	0.390	5.54
2	P1	80	2.49	4.86	0.347	4.50
2	P2	20	2.59	4.52	0.278	4.76
2	P2	40	2.59	4.36	0.482	7.08
2	P2	80	2.98	4.37	0.501	6.02
2	P3	20	2.55	4.61	0.253	4.34
2	P3	40	2.59	4.64	0.351	5.13
2	P3	80	2.51	4.93	0.531	6.85
2	TMP	20	2.47	4.98	0.354	6.68
2	TMP	40	2.57	4.93	0.403	5.80
2	TMP	80	2.63	4.82	0.367	5.17
2	P15	20	2.54	5.06	0.432	7.38
2	P15	40	2.55	5.15	0.367	5.34
2	P15	80	2.59	4.85	0.394	4.75
2	P45	20	2.57	4.76	0.337	6.13
2	P45	40	2.77	4.38	0.459	6.88
2	P45	80	2.71	4.65	0.435	4.80

Table 28. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	2.72	4.42	0.178	5.00
3		0	2.75	4.78	0.234	4.82
3	P1	20	2.82	4.64	0.213	5.57
3	P1	40	2.86	4.56	0.265	5.09
3	P1	80	2.80	4.73	0.338	5.36
3	P2	20	2.58	4.82	0.237	4.61
3	P2	40	2.98	4.22	0.336	5.74
3	P2	80	3.16	3.96	0.368	5.35
3	P3	20	2.64	4.63	0.310	4.99
3	P3	40	2.98	4.04	0.347	4.58
3	P3	80	2.81	4.55	0.363	5.65
3	TMP	20	2.88	4.44	0.255	5.66
3	TMP	40	2.89	4.40	0.298	5.19
3	TMP	80	2.82	4.40	0.444	4.66
3	P15	20	2.67	4.70	0.269	5.88
3	P15	40	2.97	4.23	0.327	5.45
3	P15	80	3.07	4.37	0.380	5.38
3	P45	20	2.85	4.67	0.241	4.96
3	P45	40	2.87	4.13	0.265	6.05
3	P45	80	2.94	4.44	0.344	4.95

Table 29. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; fiirst cutting, Ida soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	1.27	6.99	0.187	4.81
1		0	2.18	5.68	0.129	4.94
1	P1	20	2.55	5.32	0.334	6.16
1	P1	40	2.91	5.47	0.412	6.20
1	P1	80	2.08	6.07	0.532	6.70
1	P2	20	2.08	5.55	0.281	5.83
1	P2	40	2.12	5.31	0.321	4.65
1	P2	80	2.90	5.38	0.311	4.93
1	P3	20	2.46	4.53	0.283	4.19
1	P3	40	2.60	5.71	0.328	4.78
1	P3	80	2.38	5.79	0.491	6.47
1	TMP	20	1.84	5.58	0.292	6.24
1	TMP	40	2.75	5.37	0.316	5.11
1	TMP	80	2.64	5.21	0.408	5.10
1	P15	20	2.61	5.42	0.292	5.65
1	P15	40	2.76	5.15	0.301	4.93
1	P15	80	2.84	4.76	0.335	4.79
1	P45	20	2.55	5.39	0.309	5.68
1	P45	40	2.42	5.41	0.351	6.05
1	P45	80	2.35	6.99	0.375	5.00
2		0	2.12	5.01	0.219	4.81
2		0	2.27	5.09	0.242	5.40
2	P1	20	2.59	4.88	0.302	5.66
2	P1	40	2.28	5.04	0.308	5.12
2	P1	80	1.53	5.59	0.464	5.90
2	P2	20	2.88	4.66	0.293	5.00
2	P2	40	1.66	5.39	0.386	5.18
2	P2	80	1.91	5.25	0.399	4.68
2	P3	20	2.38	5.33	0.275	4.42
2	P3	40	2.53	5.19	0.279	4.43
2	P3	80	2.41	5.21	0.470	5.86
2	TMP	20	2.46	5.04	0.225	5.27
2	TMP	40	2.45	4.97	0.276	4.92
2	TMP	80	2.83	4.94	0.333	4.67
2	P15	20	2.57	4.90	0.313	5.34
2	P15	40	2.75	5.46	0.300	5.04
2	P15	80	2.65	4.77	0.541	5.80
2	P45	20	2.67	4.91	0.263	4.57
2	P45	40	2.75	5.16	0.377	4.28
2	P45	80	2.44	6.19	0.405	5.08

Table 29. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
3		0	2.43	4.82	0.239	4.92
3		0	1.89	5.50	0.228	4.51
3	P1	20	2.42	5.47	0.338	4.49
3	P1	40	2.52	5.07	0.366	5.16
3	P1	80	2.05	5.41	0.480	5.45
3	P2	20	2.36	5.38	0.349	5.12
3	P2	40	1.53	5.70	0.514	4.60
3	P2	80	2.10	5.58	0.492	4.40
3	P3	20	2.62	4.97	0.301	4.29
3	P3	40	2.86	5.07	0.417	4.76
3	P3	80	2.15	5.30	0.493	5.29
3	TMP	20	2.17	5.45	0.311	5.26
3	TMP	40	2.26	5.32	0.382	5.42
3	TMP	80	2.64	5.47	0.474	4.67
3	P15	20	2.78	5.42	0.301	5.05
3	P15	40	2.70	5.31	0.368	4.88
3	P15	80	2.69	5.09	0.445	5.10
3	P45	20	2.63	5.40	0.310	4.31
3	P45	40	2.56	4.79	0.381	4.97
3	P45	80	2.61	5.39	0.477	5.11

Table 30. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; first cutting, Primghar soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		-----%-----	
1		0	0.97	5.20	0.101	4.41
1		0	1.13	5.59	0.115	5.17
1	P1	20	1.76	5.68	0.205	5.21
1	P1	40	2.08	5.39	0.196	4.80
1	P1	80	2.33	5.20	0.388	4.76
1	P2	20	1.85	5.57	0.172	4.64
1	P2	40	2.03	5.47	0.245	4.83
1	P2	80	2.23	5.02	0.266	5.02
1	P3	20	1.66	6.19	0.188	5.26
1	P3	40	1.89	5.41	0.190	4.67
1	P3	80	2.42	4.99	0.370	6.16
1	TMP	20	1.83	5.14	0.220	5.28
1	TMP	40	1.91	5.33	0.225	5.07
1	TMP	80	2.34	4.95	0.394	6.20
1	P15	20	1.54	5.31	0.225	6.18
1	P15	40	1.93	4.72	0.236	5.61
1	P15	80	2.49	4.75	0.233	4.13
1	P45	20	1.84	5.87	0.239	4.95
1	P45	40	2.09	5.12	0.175	5.33
1	P45	80	2.13	5.55	0.266	4.51
2		0	1.11	5.55	0.129	4.39
2		0	1.07	5.25	0.134	4.83
2	P1	20	1.99	5.11	0.217	4.83
2	P1	40	2.14	5.20	0.326	5.71
2	P1	80	2.45	4.93	0.377	4.27
2	P2	20	1.67	5.25	0.190	5.38
2	P2	40	2.15	4.75	0.208	4.49
2	P2	80	2.40	4.69	0.358	5.28
2	P3	20	1.81	5.20	0.211	5.23
2	P3	40	1.97	5.65	0.261	4.34
2	P3	80	2.15	4.80	0.367	5.45
2	TMP	20	1.75	4.96	0.220	5.29
2	TMP	40	2.08	4.79	0.255	4.15
2	TMP	80	2.28	5.01	0.369	4.07
2	P15	20	1.76	5.55	0.168	4.56
2	P15	40	2.02	6.05	0.241	4.92
2	P15	80	2.27	5.07	0.328	4.25
2	P45	20	1.96	5.41	0.222	4.47
2	P45	40	2.00	4.98	0.217	4.33
2	P45	80	2.04	5.34	0.261	4.97

Table 30. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	1.25	5.42	0.168	5.25
3		0	1.09	5.09	0.130	4.71
3	P1	20	1.97	5.55	0.265	5.06
3	P1	40	2.21	5.08	0.326	5.14
3	P1	80	2.38	4.88	0.412	4.49
3	P2	20	2.14	5.43	0.253	4.96
3	P2	40	2.25	5.12	0.257	5.03
3	P2	80	2.49	4.82	0.385	4.74
3	P3	20	2.05	5.10	0.246	5.48
3	P3	40	2.24	5.09	0.307	5.26
3	P3	80	2.36	4.87	0.379	4.84
3	TMP	20	1.92	5.14	0.234	4.77
3	TMP	40	1.92	5.09	0.232	5.00
3	TMP	80	2.43	5.11	0.380	5.14
3	P15	20	1.67	5.39	0.207	5.15
3	P15	40	1.93	5.12	0.304	5.41
3	P15	80	2.29	4.39	0.399	4.94
3	P45	20	1.78	5.13	0.227	4.93
3	P45	40	2.26	4.99	0.233	5.11
3	P45	80	2.24	4.84	0.401	4.94

Table 31. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; first cutting, Webster soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	2.17	4.78	0.204	4.52
1		0	2.35	4.53	0.260	6.34
1	P1	20	2.18	4.94	0.427	6.91
1	P1	40	2.39	4.70	0.462	6.93
1	P1	80	2.45	4.80	0.460	5.97
1	P2	20	2.25	4.79	0.365	5.97
1	P2	40	2.32	4.45	0.370	5.46
1	P2	80	2.58	4.31	0.467	6.21
1	P3	20	2.42	4.28	0.427	6.96
1	P3	40	2.48	4.63	0.457	6.82
1	P3	80	2.67	4.50	0.538	6.13
1	TMP	20	2.30	4.60	0.306	5.83
1	TMP	40	2.44	5.06	0.323	5.61
1	TMP	80	2.42	4.82	0.492	5.86
1	P15	20	2.10	5.17	0.330	5.69
1	P15	40	2.30	5.10	0.376	6.02
1	P15	80	2.60	5.00	0.492	5.97
1	P45	20	2.51	4.92	0.342	6.17
1	P45	40	2.43	4.90	0.404	6.34
1	P45	80	2.48	4.56	0.433	5.25
2		0	1.85	6.44	0.213	5.06
2		0	2.07	5.05	0.238	4.95
2	P1	20	2.31	4.64	0.288	4.09
2	P1	40	2.22	4.83	0.248	3.05
2	P1	80	2.56	4.82	0.331	3.79
2	P2	20	2.25	5.04	0.240	5.07
2	P2	40	2.31	4.82	0.479	6.37
2	P2	80	2.52	4.13	0.540	5.79
2	P3	20	2.43	4.64	0.310	5.26
2	P3	40	2.27	4.63	0.443	4.96
2	P3	80	2.67	4.31	0.456	5.00
2	TMP	20	2.34	4.45	0.239	5.53
2	TMP	40	2.22	4.79	0.397	5.95
2	TMP	80	2.51	4.19	0.468	4.88
2	P15	20	2.16	4.51	0.289	4.42
2	P15	40	2.31	4.73	0.429	4.57
2	P15	80	2.29	4.72	0.408	5.41
2	P45	20	2.28	4.47	0.300	6.04
2	P45	40	2.38	4.50	0.332	4.72
2	P45	80	2.44	4.50	0.392	4.12

Table 31. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
3		0	2.23	4.96	0.204	4.95
3		0	2.08	4.95	0.207	5.25
3	P1	20	2.07	4.75	0.317	5.13
3	P1	40	2.37	4.81	0.353	5.42
3	P1	80	2.40	4.77	0.350	5.23
3	P2	20	2.54	4.66	0.274	5.22
3	P2	40	2.31	4.97	0.385	5.34
3	P2	80	2.41	4.66	0.405	5.00
3	P3	20	2.31	4.46	0.331	4.66
3	P3	40	2.40	4.80	0.317	5.09
3	P3	80	2.42	4.52	0.401	4.98
3	TMP	20	2.29	4.63	0.319	4.70
3	TMP	40	2.74	4.75	0.430	4.91
3	TMP	80	2.64	4.95	0.426	5.01
3	P15	20	2.28	4.89	0.340	5.01
3	P15	40	2.25	5.04	0.378	3.88
3	P15	80	2.54	4.66	0.411	5.34
3	P45	20	2.51	4.69	0.365	5.08
3	P45	40	2.44	4.92	0.435	5.47
3	P45	80	2.41	4.71	0.565	5.48

Table 32. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; second cutting, Clarion soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%-	
1		0	3.01	4.09	0.197	3.76
1		0	2.74	4.17	0.249	4.60
1	P1	20	2.98	4.07	0.255	4.66
1	P1	40	3.36	3.73	0.298	4.54
1	P1	80	3.45	3.70	0.387	4.62
1	P2	20	3.06	3.69	0.265	4.16
1	P2	40	3.11	4.09	0.289	4.29
1	P2	80	3.60	3.59	0.358	4.35
1	P3	20	2.96	4.23	0.272	4.61
1	P3	40	3.23	3.56	0.319	4.79
1	P3	80	3.20	4.42	0.410	4.04
1	TMP	20	2.90	4.36	0.243	4.59
1	TMP	40	3.25	4.16	0.251	4.88
1	TMP	80	3.33	3.90	0.413	4.48
1	P15	20	3.14	4.00	0.236	4.28
1	P15	40	3.13	3.81	0.313	4.46
1	P15	80	3.44	3.96	0.382	5.03
1	P45	20	3.03	3.95	0.262	4.71
1	P45	40	3.03	4.26	0.293	5.46
1	P45	80	3.12	3.74	0.444	4.40
2		0	2.73	4.20	0.180	3.94
2		0	2.57	4.05	0.180	4.35
2	P1	20	2.80	3.81	0.231	3.79
2	P1	40	3.20	3.89	0.253	4.17
2	P1	80	3.57	3.85	0.273	4.95
2	P2	20	3.43	3.93	0.205	4.62
2	P2	40	2.95	4.20	0.255	4.45
2	P2	80	3.32	3.94	0.263	4.38
2	P3	20	3.06	4.47	0.220	3.86
2	P3	40	3.02	4.14	0.289	3.74
2	P3	80	3.36	4.01	0.357	4.33
2	TMP	20	3.08	4.36	0.190	4.23
2	TMP	40	3.01	4.10	0.340	3.97
2	TMP	80	3.18	3.88	0.322	4.59
2	P15	20	2.77	4.06	0.191	4.74
2	P15	40	2.92	3.80	0.309	4.21
2	P15	80	3.49	3.70	0.379	3.92
2	P45	20	2.91	3.96	0.216	3.90
2	P45	40	3.36	4.08	0.281	3.82
2	P45	80	3.19	4.20	0.392	4.80

Table 32. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
3		0	2.50	4.12	0.150	3.84
3		0	2.75	4.02	0.102	3.95
3	P1	20	2.82	4.02	0.165	4.33
3	P1	40	3.07	4.13	0.202	4.63
3	P1	80	3.18	4.03	0.234	4.53
3	P2	20	3.08	3.96	0.145	3.91
3	P2	40	2.76	4.09	0.282	4.68
3	P2	80	3.42	3.89	0.300	3.75
3	P3	20	2.77	3.80	0.252	3.96
3	P3	40	3.11	3.79	0.291	4.31
3	P3	80	3.10	3.81	0.310	4.24
3	TMP	20	3.13	3.85	0.197	3.85
3	TMP	40	3.21	3.84	0.266	3.87
3	TMP	80	3.03	4.08	0.276	4.34
3	P15	20	3.08	4.12	0.198	4.35
3	P15	40	2.97	3.85	0.302	4.28
3	P15	80	3.20	3.61	0.374	4.48
3	P45	20	3.29	4.08	0.170	4.06
3	P45	40	3.17	3.95	0.295	3.94
3	P45	80	3.47	3.86	0.353	3.82

Table 33. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; second cutting, Ida soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	3.24	4.47	0.167	3.58
1		0	3.08	4.86	0.140	3.50
1	P1	20	3.80	3.99	0.173	3.82
1	P1	40	3.84	3.63	0.198	3.39
1	P1	80	4.10	3.95	0.347	3.90
1	P2	20	3.44	4.28	0.196	3.31
1	P2	40	3.83	4.33	0.323	3.88
1	P2	80	3.48	4.36	0.309	3.52
1	P3	20	3.68	4.09	0.212	3.65
1	P3	40	3.37	4.12	0.241	3.32
1	P3	80	3.73	4.32	0.344	3.48
1	TMP	20	3.72	4.49	0.175	3.70
1	TMP	40	3.92	3.97	0.297	3.97
1	TMP	80	3.51	4.28	0.338	3.41
1	P15	20	3.42	3.95	0.199	3.94
1	P15	40	3.87	3.98	0.246	3.66
1	P15	80	3.77	4.06	0.343	3.61
1	P45	20	3.67	4.11	0.179	3.39
1	P45	40	3.56	4.31	0.264	3.49
1	P45	80	3.86	4.62	0.376	4.22
2		0	3.10	4.48	0.143	3.19
2		0	2.96	4.85	0.158	3.37
2	P1	20	3.34	4.52	0.173	3.57
2	P1	40	3.77	4.02	0.199	3.20
2	P1	80	3.35	4.99	0.333	4.38
2	P2	20	3.93	3.70	0.226	3.71
2	P2	40	3.41	4.47	0.300	4.51
2	P2	80	3.62	4.19	0.351	3.66
2	P3	20	3.53	4.14	0.196	3.74
2	P3	40	3.62	4.24	0.298	3.93
2	P3	80	3.34	4.53	0.345	3.68
2	TMP	20	3.81	4.17	0.186	3.34
2	TMP	40	3.92	4.20	0.243	3.36
2	TMP	80	3.22	3.88	0.243	3.57
2	P15	20	3.75	4.03	0.203	3.43
2	P15	40	3.50	4.41	0.240	3.54
2	P15	80	4.08	3.23	0.297	3.61
2	P45	20	3.90	3.90	0.251	4.24
2	P45	40	3.26	4.31	0.356	4.49
2	P45	80	3.44	4.32	0.388	3.60

Table 33. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	2.91	4.52	0.207	3.41
3		0	3.14	4.64	0.139	3.57
3	P1	20	3.53	4.27	0.190	3.53
3	P1	40	3.37	4.47	0.285	3.58
3	P1	80	3.61	4.56	0.334	4.07
3	P2	20	3.26	4.70	0.211	3.54
3	P2	40	3.24	4.84	0.231	3.85
3	P2	80	3.71	4.86	0.320	3.67
3	P3	20	3.41	4.29	0.209	4.16
3	P3	40	3.55	4.42	0.255	3.79
3	P3	80	4.09	4.17	0.265	3.70
3	TMP	20	3.60	4.47	0.230	3.64
3	TMP	40	3.82	4.22	0.219	3.86
3	TMP	80	4.02	3.97	0.281	3.65
3	P15	20	4.00	3.86	0.206	3.63
3	P15	40	3.46	4.12	0.238	3.42
3	P15	80	3.80	3.98	0.264	3.35
3	P45	20	3.33	4.27	0.208	3.29
3	P45	40	3.87	4.23	0.250	3.68
3	P45	80	3.82	4.18	0.282	3.42

Table 34. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; second cutting, Primghar soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	1.40	5.12	0.102	3.29
1		0	1.46	5.08	0.095	3.05
1	P1	20	3.06	4.51	0.126	3.52
1	P1	40	3.57	4.15	0.166	3.88
1	P1	80	3.97	3.81	0.203	3.25
1	P2	20	3.40	4.06	0.124	3.64
1	P2	40	3.70	4.08	0.186	2.95
1	P2	80	3.37	4.08	0.238	3.87
1	P3	20	3.08	4.46	0.166	3.28
1	P3	40	3.20	4.11	0.178	3.05
1	P3	80	3.90	3.57	0.209	3.24
1	TMP	20	2.85	4.66	0.139	3.66
1	TMP	40	3.57	3.98	0.143	3.19
1	TMP	80	3.65	4.29	0.283	3.66
1	P15	20	2.70	4.74	0.114	3.42
1	P15	40	2.98	4.42	0.160	3.51
1	P15	80	4.07	3.75	0.196	3.79
1	P45	20	2.87	4.53	0.173	3.49
1	P45	40	3.39	4.36	0.157	3.36
1	P45	80	3.23	4.21	0.295	3.79
2		0	1.48	5.39	0.104	4.03
2		0	1.59	5.40	0.070	2.99
2	P1	20	2.93	4.45	0.147	3.40
2	P1	40	3.74	4.11	0.118	3.20
2	P1	80	3.68	3.88	0.200	3.06
2	P2	20	3.23	3.88	0.124	2.88
2	P2	40	3.29	4.33	0.156	2.84
2	P2	80	3.62	3.87	0.177	2.94
2	P3	20	2.70	4.45	0.189	3.39
2	P3	40	3.23	4.21	0.145	3.76
2	P3	80	3.14	4.17	0.246	2.96
2	TMP	20	2.98	4.34	0.149	3.08
2	TMP	40	3.07	4.59	0.155	3.02
2	TMP	80	3.40	3.98	0.281	3.42
2	P15	20	2.90	4.42	0.129	2.67
2	P15	40	3.12	3.97	0.132	2.72
2	P15	80	3.78	3.81	0.183	2.72
2	P45	20	2.33	5.19	0.137	3.33
2	P45	40	3.29	4.32	0.211	3.03
2	P45	80	3.66	3.96	0.317	3.20

Table 34. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%---------	
3		0	1.73	4.87	0.094	3.22
3		0	1.43	5.17	0.118	4.19
3	P1	20	2.84	4.36	0.197	3.69
3	P1	40	3.20	4.15	0.128	3.12
3	P1	80	3.73	3.93	0.137	2.79
3	P2	20	2.93	4.40	0.131	3.01
3	P2	40	3.66	3.70	0.107	3.15
3	P2	80	3.82	3.70	0.173	3.49
3	P3	20	3.04	4.39	0.156	2.89
3	P3	40	3.12	4.01	0.177	2.96
3	P3	80	3.81	3.84	0.178	2.59
3	TMP	20	3.04	4.19	0.126	2.61
3	TMP	40	3.68	3.93	0.145	2.72
3	TMP	80	3.44	4.15	0.141	2.76
3	P15	20	2.85	4.52	0.129	2.76
3	P15	40	2.89	4.51	0.161	2.90
3	P15	80	3.51	4.21	0.195	3.16
3	P45	20	2.76	4.28	0.131	3.67
3	P45	40	3.49	3.82	0.145	3.37
3	P45	80	3.93	3.74	0.158	2.99

Table 35. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; second cutting, Webster soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	3.10	3.97	0.207	4.24
1		0	2.90	4.31	0.213	3.64
1	P1	20	3.00	4.31	0.313	4.51
1	P1	40	3.17	4.00	0.351	4.16
1	P1	80	3.50	3.69	0.408	3.89
1	P2	20	3.30	4.12	0.282	4.01
1	P2	40	3.17	3.82	0.322	4.31
1	P2	80	3.55	3.95	0.440	4.29
1	P3	20	2.86	4.00	0.257	4.06
1	P3	40	3.02	3.63	0.266	4.03
1	P3	80	3.20	3.72	0.379	4.07
1	TMP	20	3.17	3.91	0.184	4.05
1	TMP	40	3.32	3.67	0.194	4.08
1	TMP	80	3.24	4.14	0.273	4.31
1	P15	20	2.81	3.90	0.237	4.44
1	P15	40	3.14	2.80	0.262	4.24
1	P15	80	3.35	4.02	0.339	3.96
1	P45	20	3.18	4.02	0.222	4.13
1	P45	40	3.30	3.81	0.284	4.02
1	P45	80	3.15	3.65	0.288	3.80
2		0	2.32	4.42	0.216	3.79
2		0	2.75	4.05	0.138	3.24
2	P1	20	2.67	4.22	0.195	4.09
2	P1	40	3.09	3.99	0.323	3.56
2	P1	80	3.47	3.88	0.312	3.76
2	P2	20	2.82	4.11	0.247	4.02
2	P2	40	3.04	4.44	0.250	3.92
2	P2	80	3.28	3.81	0.293	4.36
2	P3	20	2.51	4.14	0.208	3.80
2	P3	40	3.10	3.99	0.244	3.80
2	P3	80	3.30	3.91	0.397	3.84
2	TMP	20	2.88	4.10	0.171	3.55
2	TMP	40	2.99	4.14	0.194	3.53
2	TMP	80	2.98	4.16	0.325	3.87
2	P15	20	3.31	4.04	0.191	3.83
2	P15	40	2.98	4.16	0.259	3.56
2	P15	80	3.26	4.43	0.307	4.19
2	P45	20	3.15	4.01	0.231	4.49
2	P45	40	3.02	4.03	0.262	4.04
2	P45	80	2.64	3.77	0.293	4.22

Table 35. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	2.75	4.21	0.184	3.43
3		0	2.82	4.20	0.194	3.64
3	P1	20	2.86	4.40	0.151	4.15
3	P1	40	3.13	4.72	0.280	3.57
3	P1	80	3.04	4.07	0.292	3.55
3	P2	20	2.99	3.87	0.241	3.78
3	P2	40	3.00	4.29	0.288	3.85
3	P2	80	3.34	4.08	0.350	4.25
3	P3	20	3.11	3.88	0.200	3.98
3	P3	40	3.20	3.76	0.228	4.17
3	P3	80	3.14	4.04	0.280	3.58
3	TMP	20	3.06	3.90	0.178	4.10
3	TMP	40	3.11	4.26	0.211	3.93
3	TMP	80	3.22	4.07	0.188	4.46
3	P15	20	3.15	4.55	0.205	4.36
3	P15	40	3.10	4.14	0.240	3.41
3	P15	80	3.56	3.90	0.327	3.83
3	P45	20	3.07	4.20	0.184	4.27
3	P45	40	2.92	4.17	0.254	3.51
3	P45	80	3.32	4.20	0.289	3.99

Table 36. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; third cutting, Clarion soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%---	
1		0	4.05	3.79	0.149	3.21
1		0	4.05	3.80	0.162	3.40
1	P1	20	4.83	3.12	0.144	4.42
1	P1	40	4.72	3.19	0.166	5.04
1	P1	80	5.04	3.66	0.301	4.61
1	P2	20	4.94	3.58	0.194	3.90
1	P2	40	5.14	2.70	0.153	4.54
1	P2	80	4.83	3.19	0.259	4.42
1	P3	20	4.38	3.39	0.138	4.14
1	P3	40	4.50	3.61	0.358	4.32
1	P3	80	5.15	3.15	0.243	4.14
1	TMP	20	4.54	2.65	0.163	4.19
1	TMP	40	4.77	2.68	0.235	4.01
1	TMP	80	4.90	3.26	0.246	3.81
1	P15	20	4.36	3.13	0.076	4.17
1	P15	40	4.73	3.43	0.147	4.50
1	P15	80	4.80	3.12	0.197	4.24
1	P45	20	4.59	3.31	0.102	4.57
1	P45	40	5.46	2.97	0.129	4.45
1	P45	80	4.84	3.35	0.228	4.27
2		0	3.83	3.27	0.160	4.14
2		0	4.06	3.02	0.172	4.63
2	P1	20	4.65	2.97	0.191	4.36
2	P1	40	4.58	2.91	0.218	4.25
2	P1	80	5.12	2.81	0.292	4.07
2	P2	20	4.72	2.64	0.200	4.53
2	P2	40	4.70	2.96	0.246	4.62
2	P2	80	4.96	2.94	0.329	4.49
2	P3	20	3.95	3.40	0.227	4.44
2	P3	40	4.60	2.97	0.271	4.59
2	P3	80	4.83	2.45	0.300	4.06
2	TMP	20	4.37	2.85	0.195	4.12
2	TMP	40	4.61	2.21	0.191	3.87
2	TMP	80	4.65	2.69	0.200	3.71
2	P15	20	4.65	3.13	0.133	3.85
2	P15	40	4.39	3.68	0.190	4.91
2	P15	80	4.73	2.95	0.357	4.40
2	P45	20	4.66	3.12	0.201	4.24
2	P45	40	4.63	2.55	0.243	4.63
2	P45	80	4.81	2.68	0.323	4.36

Table 36. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%		
3		0	3.51	3.02	0.208	4.67
3		0	3.58	2.87	0.209	4.87
3	P1	20	4.37	2.97	0.203	5.05
3	P1	40	4.30	2.68	0.228	4.60
3	P1	80	4.20	2.98	0.216	3.74
3	P2	20	4.33	2.45	0.331	4.90
3	P2	40	4.34	3.22	0.256	3.74
3	P2	80	4.60	2.74	0.344	4.55
3	P3	20	4.30	2.53	0.212	4.67
3	P3	40	4.72	2.94	0.284	4.96
3	P3	80	4.70	2.79	0.281	4.02
3	TMP	20	4.17	2.69	0.291	4.36
3	TMP	40	4.40	2.53	0.213	4.04
3	TMP	80	4.41	2.62	0.239	4.72
3	P15	20	4.24	2.51	0.207	4.57
3	P15	40	4.52	2.89	0.287	4.92
3	P15	80	4.72	2.42	0.283	4.32
3	P45	20	4.43	2.53	0.203	4.64
3	P45	40	4.21	3.09	0.285	4.57
3	P45	80	4.39	2.98	0.308	4.25

Table 37. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; third cutting, Ida soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
1		0	3.90	3.97	0.136	4.63
1		0	4.07	2.88	0.178	4.11
1	P1	20	4.73	3.16	0.157	4.06
1	P1	40	4.27	3.30	0.201	4.49
1	P1	80	4.70	4.69	0.156	4.14
1	P2	20	3.77	3.80	0.190	3.78
1	P2	40	4.50	3.25	0.262	4.57
1	P2	80	3.91	3.80	0.235	4.02
1	P3	20	4.39	3.42	0.187	4.13
1	P3	40	3.27	4.00	0.216	4.13
1	P3	80	4.12	3.12	0.240	4.31
1	TMP	20	3.96	4.54	0.179	5.60
1	TMP	40	4.33	3.22	0.194	4.13
1	TMP	80	4.44	3.28	0.299	4.22
1	P15	20	4.22	3.73	0.125	4.00
1	P15	40	4.50	4.49	0.173	3.93
1	P15	80	4.22	4.16	0.258	4.18
1	P45	20	3.91	4.59	0.200	4.10
1	P45	40	3.80	4.43	0.204	4.34
1	P45	80	4.59	4.45	0.307	4.07
2		0	3.85	3.67	0.156	4.09
2		0	3.84	3.73	0.104	3.52
2	P1	20	3.40	3.41	0.204	4.06
2	P1	40	4.29	2.87	0.214	3.93
2	P1	80	3.38	3.42	0.246	4.36
2	P2	20	3.97	2.59	0.203	4.13
2	P2	40	4.26	2.63	0.221	4.17
2	P2	80	4.28	2.90	0.238	3.87
2	P3	20	4.17	3.31	0.181	4.17
2	P3	40	3.76	3.78	0.218	4.08
2	P3	80	3.72	3.90	0.268	4.05
2	TMP	20	4.00	3.50	0.266	4.79
2	TMP	40	4.25	2.94	0.218	4.11
2	TMP	80	4.28	3.57	0.318	4.11
2	P15	20	4.04	3.22	0.207	4.06
2	P15	40	3.71	3.76	0.235	3.93
2	P15	80	4.22	3.12	0.275	3.97
2	P45	20	4.22	3.10	0.207	4.25
2	P45	40	3.65	3.68	0.236	4.16
2	P45	80	3.55	4.02	0.263	3.87

Table 37. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%----------	
3		0	2.88	3.40	0.208	4.45
3		0	3.57	3.73	0.195	3.44
3	P1	20	3.82	2.88	0.187	3.71
3	P1	40	3.22	3.70	0.233	3.87
3	P1	80	3.62	2.98	0.325	4.19
3	P2	20	3.33	3.86	0.183	4.17
3	P2	40	3.55	2.80	0.204	4.08
3	P2	80	3.60	3.30	0.264	3.94
3	P3	20	3.68	2.80	0.193	3.93
3	P3	40	3.50	2.63	0.165	3.83
3	P3	80	4.07	2.30	0.320	4.35
3	TMP	20	3.51	3.47	0.203	4.43
3	TMP	40	3.92	3.36	0.199	4.24
3	TMP	80	4.00	3.15	0.239	3.96
3	P15	20	4.14	3.06	0.234	4.40
3	P15	40	3.48	3.34	0.241	3.97
3	P15	80	3.77	3.24	0.263	3.99
3	P45	20	3.36	3.44	0.216	4.10
3	P45	40	3.57	3.65	0.144	3.87
3	P45	80	3.85	2.92	0.292	4.31

Table 38. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; third cutting, Primghar soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		-----%-----	
1		0	1.42	5.22	0.080	3.93
1		0	1.24	5.66	0.094	4.17
1	P1	20	3.79	4.15	0.113	3.66
1	P1	40	4.44	3.50	0.086	3.77
1	P1	80	4.66	3.80	0.164	3.37
1	P2	20	3.89	4.32	0.138	3.99
1	P2	40	4.65	3.74	0.154	3.66
1	P2	80	4.81	3.50	0.138	3.61
1	P3	20	3.45	5.36	0.158	3.84
1	P3	40	3.76	4.61	0.175	3.66
1	P3	80	4.71	3.57	0.191	4.25
1	TMP	20	3.50	4.06	0.076	3.56
1	TMP	40	4.22	3.64	0.097	4.03
1	TMP	80	4.63	3.65	0.224	3.91
1	P15	20	3.60	3.57	0.111	3.64
1	P15	40	3.79	3.46	0.170	4.52
1	P15	80	4.90	2.55	0.203	4.49
1	P45	20	3.63	3.66	0.127	4.31
1	P45	40	4.28	1.93	0.163	4.35
1	P45	80	3.97	3.37	0.266	4.21
2		0	1.45	4.62	0.085	3.74
2		0	1.38	4.70	0.093	4.51
2	P1	20	3.79	3.24	0.127	4.61
2	P1	40	4.07	2.84	0.123	4.10
2	P1	80	4.56	2.91	0.161	3.96
2	P2	20	3.47	3.68	0.118	4.40
2	P2	40	4.12	3.14	0.183	4.19
2	P2	80	4.53	2.98	0.258	4.00
2	P3	20	3.44	3.58	0.143	4.12
2	P3	40	4.17	3.74	0.205	4.25
2	P3	80	4.73	3.16	0.230	4.25
2	TMP	20	3.83	3.51	0.144	4.24
2	TMP	40	4.32	3.16	0.153	4.46
2	TMP	80	4.84	3.02	0.220	3.80
2	P15	20	3.36	3.09	0.184	4.30
2	P15	40	3.78	2.93	0.217	4.59
2	P15	80	4.40	2.84	0.212	4.21
2	P45	20	3.22	3.26	0.147	4.35
2	P45	40	4.16	3.29	0.175	4.07
2	P45	80	4.30	2.86	0.209	4.18

Table 38. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	1.30	4.31	0.068	3.77
3		0	1.15	4.20	0.072	4.25
3	P1	20	3.61	3.39	0.099	3.86
3	P1	40	3.65	2.87	0.192	4.31
3	P1	80	4.11	2.80	0.179	4.10
3	P2	20	2.88	6.68	0.118	3.96
3	P2	40	3.23	2.91	0.116	4.55
3	P2	80	4.28	2.58	0.176	3.97
3	P3	20	3.01	3.75	0.119	3.93
3	P3	40	4.12	3.23	0.140	4.33
3	P3	80	4.29	2.85	0.181	4.47
3	TMP	20	3.35	3.96	0.150	3.83
3	TMP	40	3.81	3.63	0.160	4.02
3	TMP	80	4.20	3.22	0.236	4.06
3	P15	20	3.01	4.08	0.113	3.71
3	P15	40	3.65	4.10	0.203	4.40
3	P15	80	4.33	3.30	0.188	3.84
3	P45	20	3.00	4.22	0.143	3.85
3	P45	40	3.70	3.66	0.137	3.78
3	P45	80	3.76	3.56	0.200	4.21

Table 39. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; third cutting, Webster soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		--------%--------	
1		0	4.54	3.50	0.173	4.00
1		0	4.54	3.60	0.119	4.10
1	P1	20	5.33	3.51	0.152	4.83
1	P1	40	5.04	3.26	0.281	4.54
1	P1	80	5.18	3.42	0.540	3.93
1	P2	20	4.98	3.35	0.209	4.53
1	P2	40	4.88	2.75	0.185	4.34
1	P2	80	5.46	3.00	0.210	4.43
1	P3	20	4.55	3.41	0.180	4.49
1	P3	40	5.04	2.94	0.264	4.33
1	P3	80	3.18	2.87	0.210	4.17
1	TMP	20	4.93	3.67	0.162	4.17
1	TMP	40	5.50	3.29	0.214	3.96
1	TMP	80	4.57	3.58	0.307	4.51
1	P15	20	4.49	2.43	0.221	4.61
1	P15	40	5.05	2.92	0.188	4.43
1	P15	80	4.95	2.64	0.233	3.64
1	P45	20	4.89	2.95	0.226	4.16
1	P45	40	5.18	2.74	0.230	4.12
1	P45	80	4.88	2.68	0.298	4.46
2		0	3.94	3.27	0.211	4.56
2		0	4.05	3.31	0.212	4.40
2	P1	20	4.31	3.18	0.252	4.36
2	P1	40	4.88	2.69	0.340	4.29
2	P1	80	5.02	2.93	0.410	4.40
2	P2	20	4.02	2.76	0.220	4.84
2	P2	40	4.81	2.55	0.292	4.00
2	P2	80	4.73	2.63	0.344	3.57
2	P3	20	4.53	3.15	0.212	3.73
2	P3	40	4.47	2.83	0.257	3.67
2	P3	80	5.04	2.30	0.311	4.51
2	TMP	20	4.41	3.04	0.219	3.62
2	TMP	40	4.87	2.82	0.253	4.22
2	TMP	80	5.00	2.39	0.348	4.35
2	P15	20	4.57	3.10	0.274	4.72
2	P15	40	4.88	2.54	0.249	4.52
2	P15	80	5.08	2.32	0.321	4.83
2	P45	20	4.30	2.54	0.229	3.97
2	P45	40	4.65	2.66	0.244	4.17
2	P45	80	5.14	2.62	0.323	3.98

Table 39. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%---------	
3		0	4.31	2.70	0.223	4.42
3		0	3.84	3.31	0.209	4.47
3	P1	20	3.93	3.25	0.265	4.77
3	P1	40	4.58	2.67	0.263	3.96
3	P1	80	4.41	2.87	0.300	3.45
3	P2	20	4.62	3.07	0.297	4.31
3	P2	40	4.28	2.94	0.263	4.11
3	P2	80	3.29	2.86	0.289	3.57
3	P3	20	4.43	2.97	0.246	4.37
3	P3	40	4.02	3.09	0.247	3.71
3	P3	80	4.04	2.66	0.368	4.01
3	TMP	20	4.02	2.53	0.225	4.08
3	TMP	40	4.62	2.67	0.246	3.92
3	TMP	80	4.45	3.09	0.357	3.93
3	P15	20	4.14	2.99	0.187	4.30
3	P15	40	4.57	3.12	0.251	3.85
3	P15	80	4.70	2.31	0.310	3.71
3	P45	20	4.52	2.66	0.201	4.29
3	P45	40	4.72	2.65	0.312	4.11
3	P45	80	4.61	2.23	0.332	4.19

Table 40. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; fourth cutting, Clarion soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	3.42	3.13	0.164	3.61
1		0	3.12	3.40	0.196	4.37
1	P1	20	5.23	3.03	0.100	3.46
1	P1	40	6.21	3.50	0.121	3.36
1	P1	80	4.88	2.81	0.241	3.58
1	P2	20	5.48	2.70	0.115	3.36
1	P2	40	5.24	2.71	0.179	3.74
1	P2	80	4.66	2.86	0.266	4.12
1	P3	20	5.38	2.74	0.118	4.12
1	P3	40	4.61	2.98	0.220	3.92
1	P3	80	5.28	2.47	0.280	3.84
1	TMP	20	4.72	2.97	0.153	3.50
1	TMP	40	4.73	2.88	0.217	3.81
1	TMP	80	5.62	2.70	0.304	3.86
1	P15	20	4.75	2.80	0.163	3.55
1	P15	40	5.28	2.80	0.212	3.65
1	P15	80	5.25	2.75	0.292	3.58
1	P45	20	4.24	3.43	0.201	3.64
1	P45	40	4.85	2.81	0.227	3.62
1	P45	80	5.90	2.67	0.245	3.58
2		0	3.50	3.07	0.191	4.12
2		0	4.10	2.54	0.189	4.22
2	P1	20	4.81	2.88	0.196	3.97
2	P1	40	4.89	2.45	0.199	3.65
2	P1	80	4.90	2.60	0.304	3.78
2	P2	20	4.65	2.55	0.223	4.37
2	P2	40	5.22	2.04	0.236	3.75
2	P2	80	4.94	2.52	0.310	3.74
2	P3	20	4.44	2.23	0.217	4.06
2	P3	40	4.66	2.66	0.254	4.02
2	P3	80	5.04	2.10	0.293	3.90
2	TMP	20	4.88	2.28	0.200	3.60
2	TMP	40	4.95	2.38	0.245	3.85
2	TMP	80	5.39	3.02	0.335	4.36
2	P15	20	4.50	3.01	0.144	3.81
2	P15	40	4.91	2.87	0.179	3.90
2	P15	80	4.81	2.81	0.221	3.86
2	P45	20	5.13	2.78	0.152	3.69
2	P45	40	4.91	2.81	0.206	4.01
2	P45	80	5.44	2.51	0.278	4.00

Table 40. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
3		0	3.93	3.25	0.174	3.75
3		0	4.12	3.37	0.158	3.55
3	P1	20	5.02	2.02	0.191	3.58
3	P1	40	5.55	2.69	0.151	4.04
3	P1	80	4.61	3.07	0.250	3.84
3	P2	20	4.64	2.71	0.148	3.99
3	P2	40	4.81	2.86	0.147	4.07
3	P2	80	4.76	2.81	0.216	4.29
3	P3	20	4.48	3.29	0.183	3.71
3	P3	40	4.83	2.95	0.220	3.92
3	P3	80	4.80	2.84	0.274	4.10
3	TMP	20	4.80	2.22	0.228	3.93
3	TMP	40	4.45	2.92	0.240	3.56
3	TMP	80	5.16	1.82	0.335	4.05
3	P15	20	4.62	1.94	0.277	3.77
3	P15	40	4.80	2.67	0.220	3.66
3	P15	80	5.11	2.84	0.251	3.84
3	P45	20	4.78	2.83	0.177	3.59
3	P45	40	4.86	2.86	0.242	4.09
3	P45	80	4.52	2.80	0.309	3.70

Table 41. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; fourth cutting, Ida soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	4.08	3.25	0.197	3.32
1		0	3.14	3.56	0.215	3.45
1	P1	20	5.01	2.45	0.233	3.45
1	P1	40	4.75	2.90	0.226	3.16
1	P1	80	5.31	2.71	0.253	2.60
1	P2	20	3.64	3.07	0.220	3.32
1	P2	40	3.67	2.58	0.214	3.20
1	P2	80	5.17	2.58	0.262	2.65
1	P3	20	4.70	2.61	0.178	2.51
1	P3	40	3.76	3.64	0.193	2.77
1	P3	80	4.65	3.10	0.230	2.48
1	TMP	20	3.84	3.61	0.192	3.16
1	TMP	40	4.24	3.21	0.188	3.28
1	TMP	80	4.11	3.49	0.223	2.50
1	P15	20	4.11	3.21	0.228	3.53
1	P15	40	4.44	3.02	0.248	3.32
1	P15	80	4.79	2.44	0.268	3.07
1	P45	20	4.03	3.46	0.208	3.64
1	P45	40	4.03	3.09	0.166	2.61
1	P45	80	4.71	2.93	0.293	2.96
2		0	2.94	3.55	0.134	2.97
2		0	3.66	3.03	0.131	2.90
2	P1	20	4.11	3.44	0.156	3.09
2	P1	40	4.22	2.52	0.185	2.34
2	P1	80	4.48	3.66	0.268	2.98
2	P2	20	4.38	2.76	0.200	3.64
2	P2	40	5.01	2.72	0.148	2.55
2	P2	80	5.11	2.34	0.224	3.08
2	P3	20	4.99	3.31	0.155	2.60
2	P3	40	4.16	3.68	0.177	2.89
2	P3	80	5.00	3.23	0.227	2.96
2	TMP	20	4.02	3.78	0.218	3.21
2	TMP	40	4.93	2.79	0.207	2.85
2	TMP	80	5.45	2.86	0.214	2.31
2	P15	20	4.45	3.11	0.188	2.68
2	P15	40	4.51	3.17	0.263	3.49
2	P15	80	4.44	2.98	0.278	2.63
2	P45	20	4.24	3.39	0.347	3.39
2	P45	40	4.44	3.07	0.219	3.02
2	P45	80	4.59	3.50	0.247	2.52

Table 41. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	2.27	3.87	0.143	2.42
3		0	3.11	3.73	0.111	3.12
3	P1	20	4.11	3.20	0.185	2.40
3	P1	40	2.45	2.40	0.287	2.77
3	P1	80	4.73	3.27	0.270	2.89
3	P2	20	4.53	4.24	0.151	3.58
3	P2	40	3.35	4.40	0.216	2.90
3	P2	80	4.53	3.20	0.304	3.52
3	P3	20	4.85	2.96	0.167	2.56
3	P3	40	4.28	3.39	0.270	3.42
3	P3	80	4.27	3.24	0.179	3.16
3	TMP	20	3.88	3.88	0.232	3.16
3	TMP	40	4.10	3.53	0.183	2.40
3	TMP	80	4.66	2.99	0.257	2.84
3	P15	20	4.10	3.64	0.217	3.12
3	P15	40	4.15	3.44	0.238	3.08
3	P15	80	4.09	3.51	0.412	2.61
3	P45	20	4.46	3.32	0.170	2.72
3	P45	40	3.98	3.53	0.168	2.50
3	P45	80	4.50	2.89	0.252	2.85

Table 42. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; fourth cutting, Primghar soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	1.24	3.65	0.093	3.23
1		0	1.06	4.33	0.108	3.61
1	P1	20	3.87	2.74	0.141	2.70
1	P1	40	3.88	2.56	0.194	2.38
1	P1	80	4.56	2.78	0.224	1.81
1	P2	20	3.42	3.17	0.130	2.77
1	P2	40	4.05	2.69	0.193	2.26
1	P2	80	4.23	3.18	0.253	2.44
1	P3	20	2.58	3.61	0.163	3.16
1	P3	40	3.67	2.81	0.191	2.77
1	P3	80	4.01	3.20	0.228	2.14
1	TMP	20	2.86	4.30	0.157	3.18
1	TMP	40	4.28	3.14	0.188	2.53
1	TMP	80	4.25	3.32	0.253	2.31
1	P15	20	3.18	3.57	0.135	3.27
1	P15	40	3.48	3.22	0.165	2.43
1	P15	80	3.94	2.80	0.244	2.23
1	P45	20	2.67	3.78	0.148	3.02
1	P45	40	3.65	3.40	0.196	2.45
1	P45	80	4.29	3.02	0.243	2.24
2		0	1.46	4.80	0.111	4.18
2		0	1.26	4.85	0.092	3.07
2	P1	20	3.48	3.65	0.130	2.76
2	P1	40	4.14	3.27	0.205	2.10
2	P1	80	4.95	2.73	0.253	2.11
2	P2	20	2.98	4.04	0.158	2.96
2	P2	40	3.94	3.31	0.198	2.36
2	P2	80	4.21	2.84	0.183	1.60
2	P3	20	3.19	3.77	0.109	2.14
2	P3	40	3.85	3.24	0.134	1.70
2	P3	80	4.42	3.18	0.181	1.77
2	TMP	20	3.02	4.16	0.127	2.76
2	TMP	40	3.92	3.35	0.121	1.82
2	TMP	80	1.46	2.14	0.153	2.33
2	P15	20	3.05	4.20	0.110	2.47
2	P15	40	3.92	3.38	0.153	2.35
2	P15	80	4.51	2.73	0.172	1.54
2	P45	20	3.12	4.70	0.090	2.53
2	P45	40	4.76	3.50	0.085	1.37
2	P45	80	4.49	2.98	0.161	1.62

Table 42. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
3		0	1.20	4.22	0.101	3.33
3		0	1.07	4.73	0.110	3.84
3	P1	20	2.91	3.78	0.138	2.57
3	P1	40	4.18	2.98	0.174	2.34
3	P1	80	3.97	2.90	0.243	2.04
3	P2	20	2.97	4.11	0.157	2.92
3	P2	40	3.23	3.54	0.215	2.77
3	P2	80	4.44	3.05	0.213	2.14
3	P3	20	2.72	3.68	0.115	2.59
3	P3	40	3.46	2.71	0.133	1.86
3	P3	80	4.39	2.84	0.193	1.93
3	TMP	20	2.84	2.57	0.117	2.85
3	TMP	40	3.68	3.34	0.180	2.15
3	TMP	80	4.12	3.27	0.183	1.84
3	P15	20	2.97	4.02	0.124	2.67
3	P15	40	3.22	2.80	0.185	2.64
3	P15	80	3.77	3.33	0.226	1.92
3	P45	20	2.66	3.54	0.109	2.74
3	P45	40	3.75	3.27	0.131	1.82
3	P45	80	3.91	2.89	0.154	1.82

Table 43. Effect of sources and levels of P on dry matter yield, and N, P, and K content of four cuttings of ryegrass grown on four soils in a pot experiment; fourth cutting, Webster soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	3.26	3.43	0.189	3.14
1		0	3.45	3.37	0.216	3.35
1	P1	20	4.96	2.96	0.266	3.33
1	P1	40	4.85	2.28	0.336	3.26
1	P1	80	5.21	2.81	0.377	3.06
1	P2	20	5.25	2.80	0.188	2.65
1	P2	40	5.53	2.60	0.257	2.94
1	P2	80	5.77	2.23	0.284	2.61
1	P3	20	4.83	2.77	0.240	3.38
1	P3	40	5.00	2.74	0.290	3.21
1	P3	80	4.41	2.37	0.413	3.35
1	TMP	20	5.01	2.60	0.234	3.12
1	TMP	40	5.42	2.54	0.256	2.77
1	TMP	80	4.53	2.63	0.346	3.03
1	P15	20	4.20	2.95	0.253	3.00
1	P15	40	5.16	2.53	0.270	2.68
1	P15	80	4.77	2.62	0.372	3.02
1	P45	20	4.71	2.88	0.224	2.96
1	P45	40	4.72	2.10	0.265	2.66
1	P45	80	4.94	2.64	0.365	2.90
2		0	3.23	3.33	0.156	2.76
2		0	3.41	3.22	0.169	3.10
2	P1	20	4.99	3.00	0.161	3.23
2	P1	40	4.98	2.68	0.261	2.98
2	P1	80	4.87	3.16	0.262	3.13
2	P2	20	5.47	2.80	0.181	2.84
2	P2	40	5.07	2.55	0.184	2.87
2	P2	80	5.40	2.53	0.321	2.82
2	P3	20	4.56	3.36	0.182	3.00
2	P3	40	5.37	2.71	0.209	2.69
2	P3	80	5.04	2.47	0.224	2.21
2	TMP	20	4.87	2.54	0.200	3.11
2	TMP	40	5.07	2.76	0.219	2.61
2	TMP	80	5.18	2.86	0.331	3.02
2	P15	20	4.35	2.90	0.190	2.77
2	P15	40	5.04	2.33	0.224	2.62
2	P15	80	5.32	2.46	0.258	2.28
2	P45	20	4.84	2.43	0.218	3.49
2	P45	40	5.24	2.51	0.203	2.35
2	P45	80	5.36	2.46	0.307	2.78

Table 43. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	3.23	3.22	0.169	3.19
3		0	3.36	3.06	0.156	2.95
3	P1	20	4.56	2.81	0.112	3.20
3	P1	40	4.72	2.95	0.250	3.29
3	P1	80	5.10	2.31	0.270	3.18
3	P2	20	4.59	2.82	0.162	2.41
3	P2	40	4.85	2.74	0.222	2.75
3	P2	80	6.11	2.29	0.228	2.78
3	P3	20	4.41	2.70	0.179	3.34
3	P3	40	4.77	2.69	0.233	3.11
3	P3	80	4.37	3.37	0.293	2.60
3	TMP	20	4.54	2.90	0.213	2.92
3	TMP	40	4.94	2.40	0.258	2.92
3	TMP	80	4.46	2.92	0.323	3.00
3	P15	20	4.43	2.71	0.180	2.60
3	P15	40	4.97	3.10	0.213	2.42
3	P15	80	4.99	2.69	0.256	2.20
3	P45	20	4.31	2.97	0.222	2.08
3	P45	40	4.44	2.81	0.313	2.81
3	P45	80	4.86	2.67	0.313	2.66

Table 44. Effect of sources and levels of P on dry matter yield, and N, P, and K content of corn grown on four soils in a pot experiment; Clarion soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%---	
1		0	0.902	1.65	0.135	2.57
1		0	1.004	1.62	0.102	2.09
1	P1	20	1.109	1.60	0.132	2.27
1	P1	40	1.152	1.50	0.155	2.38
1	P1	80	1.220	1.47	0.171	2.29
1	P2	20	0.949	1.59	0.130	2.25
1	P2	40	1.295	1.49	0.117	2.32
1	P2	80	1.300	1.38	0.174	2.52
1	P3	20	1.157	1.63	0.115	2.09
1	P3	40	1.335	1.41	0.161	2.27
1	P3	80	1.455	1.46	0.172	2.19
1	TMP	20	1.023	1.87	0.142	2.51
1	TMP	40	1.165	1.69	0.164	2.31
1	TMP	80	1.367	1.53	0.176	2.02
1	P15	20	1.260	1.70	0.135	2.00
1	P15	40	1.332	1.50	0.145	2.36
1	P15	80	1.399	1.44	0.226	2.38
1	P45	20	1.291	1.61	0.131	2.20
1	P45	40	1.320	1.52	0.164	2.36
1	P45	80	1.371	1.48	0.171	2.02
2		0	1.248	1.50	0.102	2.15
2		0	0.966	1.31	0.116	2.13
2	P1	20	1.261	1.58	0.101	2.22
2	P1	40	1.380	1.41	0.142	2.00
2	P1	80	1.596	1.37	0.200	2.25
2	P2	20	1.143	1.67	0.137	2.32
2	P2	40	1.391	1.44	0.132	2.25
2	P2	80	1.405	1.41	0.155	2.31
2	P3	20	1.039	1.87	0.130	2.20
2	P3	40	1.310	1.35	0.144	2.13
2	P3	80	1.400	1.53	0.166	2.19
2	TMP	20	1.152	1.56	0.128	2.18
2	TMP	40	1.283	1.45	0.147	2.24
2	TMP	80	1.516	1.28	0.181	2.13
2	P15	20	1.200	1.69	0.133	2.30
2	P15	40	1.358	1.56	0.156	2.15
2	P15	80	1.400	1.49	0.210	2.16
2	P45	20	1.183	1.72	0.104	2.21
2	P45	40	1.499	1.52	0.141	2.31
2	P45	80	1.510	1.51	0.179	2.19

Table 44. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	1.182	1.40	0.114	2.32
3		0	1.200	1.39	0.123	2.48
3	P1	20	1.253	1.59	0.122	2.25
3	P1	40	1.216	1.50	0.133	2.23
3	P1	80	1.260	1.58	0.175	2.18
3	P2	20	1.105	1.49	0.143	2.41
3	P2	40	1.349	1.41	0.144	2.33
3	P2	80	1.488	1.39	0.177	2.25
3	P3	20	1.212	1.71	0.136	2.35
3	P3	40	1.358	1.45	0.143	2.18
3	P3	80	1.400	1.40	0.199	2.19
3	TMP	20	1.397	1.65	0.114	2.35
3	TMP	40	1.354	1.51	0.148	2.25
3	TMP	80	1.447	1.41	0.166	2.20
3	P15	20	1.252	1.55	0.144	2.24
3	P15	40	1.384	1.35	0.158	2.06
3	P15	80	1.425	1.35	0.217	2.18
3	P45	20	1.389	1.69	0.122	2.09
3	P45	40	1.247	1.55	0.166	2.21
3	P45	80	1.456	1.48	0.229	2.15

Table 45. Effect of sources and levels of P on dry matter yield, and N, P, and K content of corn grown on four soils in a pot experiment; Ida soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%	
1		0	1.153	1.63	0.092	0.75
1		0	1.325	1.58	0.085	0.85
1	P1	20	1.495	1.46	0.094	0.90
1	P1	40	1.530	1.50	0.116	1.07
1	P1	80	1.404	1.48	0.135	1.01
1	P2	20	1.305	1.58	0.126	1.19
1	P2	40	1.489	1.53	0.123	1.06
1	P2	80	1.495	1.67	0.134	1.18
1	P3	20	1.292	1.59	0.117	1.16
1	P3	40	1.375	1.63	0.128	1.11
1	P3	80	1.405	1.68	0.143	1.10
1	TMP	20	1.338	1.51	0.109	1.15
1	TMP	40	1.440	1.45	0.134	1.04
1	TMP	80	1.545	1.47	0.164	1.13
1	P15	20	1.247	1.58	0.116	0.95
1	P15	40	1.300	1.57	0.125	1.01
1	P15	80	1.464	1.47	0.151	1.05
1	P45	20	1.400	1.56	0.124	1.14
1	P45	40	1.515	1.54	0.137	0.99
1	P45	80	1.555	1.46	0.158	0.93
2		0	1.317	1.72	0.106	0.98
2		0	1.361	1.72	0.090	1.26
2	P1	20	1.616	1.41	0.115	1.04
2	P1	40	1.425	1.51	0.112	0.92
2	P1	80	1.485	1.72	0.140	0.96
2	P2	20	1.535	1.64	0.090	0.83
2	P2	40	1.500	1.51	0.115	1.00
2	P2	80	1.510	1.60	0.142	1.05
2	P3	20	1.418	1.53	0.118	1.01
2	P3	40	1.391	1.45	0.117	1.34
2	P3	80	1.664	1.45	0.136	0.89
2	TMP	20	1.557	1.52	0.102	1.16
2	TMP	40	1.520	1.48	0.129	1.10
2	TMP	80	1.579	1.48	0.141	1.06
2	P15	20	1.387	1.51	0.115	1.05
2	P15	40	1.428	1.48	0.141	1.13
2	P15	80	1.635	1.45	0.151	0.96
2	P45	20	1.521	1.55	0.117	0.95
2	P45	40	1.451	1.48	0.120	1.15
2	P45	80	1.721	1.42	0.145	0.98

Table 45. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%		---------
3		0	1.319	1.65	0.111	1.02
3		0	1.300	1.63	0.101	1.18
3	P1	20	1.343	1.55	0.128	1.10
3	P1	40	1.348	1.52	0.144	1.05
3	P1	80	1.599	1.41	0.151	1.06
3	P2	20	1.240	1.65	0.120	1.19
3	P2	40	1.300	1.48	0.153	1.21
3	P2	80	1.483	1.40	0.160	1.15
3	P3	20	1.300	1.65	0.153	1.14
3	P3	40	1.477	1.50	0.165	1.22
3	P3	80	1.630	1.46	0.177	1.05
3	TMP	20	1.285	1.58	0.126	1.29
3	TMP	40	1.470	1.48	0.133	1.09
3	TMP	80	1.471	1.40	0.146	1.12
3	P15	20	1.533	1.65	0.141	1.10
3	P15	40	1.748	1.50	0.175	1.06
3	P15	80	1.600	1.41	0.197	1.03
3	P45	20	1.468	1.59	0.143	1.21
3	P45	40	1.455	1.50	0.188	1.03
3	P45	80	1.667	1.41	0.184	1.08

Table 46. Effect of sources and levels of P on dry matter yield, and N, P, and K content of corn grown on four soils in a pot experiment; Primghar soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
1		0	1.338	2.36	0.109	1.46
1		0	1.164	2.18	0.082	1.54
1	P1	20	1.201	2.30	0.110	1.48
1	P1	40	1.300	2.10	0.114	1.12
1	P1	80	1.368	2.04	0.178	1.10
1	P2	20	1.281	2.34	0.125	1.55
1	P2	40	1.409	2.32	0.132	1.41
1	P2	80	1.553	2.04	0.156	1.18
1	P3	20	1.451	2.31	0.114	1.23
1	P3	40	1.468	2.13	0.121	1.29
1	P3	80	1.878	1.96	0.169	1.19
1	TMP	20	1.187	2.43	0.106	1.33
1	TMP	40	1.473	2.34	0.135	1.30
1	TMP	80	1.725	2.09	0.154	1.20
1	P15	20	1.260	2.47	0.148	1.36
1	P15	40	1.410	2.42	0.159	1.33
1	P15	80	1.664	2.01	0.175	1.30
1	P45	20	1.421	2.40	0.105	1.35
1	P45	40	1.418	2.35	0.147	1.25
1	P45	80	1.572	2.01	0.153	1.34
2		0	0.896	2.57	0.114	1.93
2		0	1.142	2.29	0.094	1.46
2	P1	20	1.291	2.48	0.112	1.43
2	P1	40	1.549	2.17	0.135	1.23
2	P1	80	1.552	2.09	0.156	1.20
2	P2	20	1.461	2.42	0.124	1.38
2	P2	40	1.625	2.21	0.132	1.43
2	P2	80	1.676	2.22	0.131	1.14
2	P3	20	1.510	2.31	0.114	1.29
2	P3	40	1.692	2.19	0.138	1.24
2	P3	80	1.477	2.10	0.176	1.05
2	TMP	20	1.399	2.41	0.100	1.11
2	TMP	40	1.500	2.37	0.149	1.03
2	TMP	80	1.787	2.13	0.148	1.37
2	P15	20	1.334	2.48	0.126	1.35
2	P15	40	1.555	2.27	0.145	1.31
2	P15	80	1.780	2.05	0.159	1.10
2	P45	20	1.484	2.30	0.109	1.12
2	P45	40	1.424	2.29	0.133	1.26
2	P45	80	1.762	1.96	0.144	1.15

Table 46. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%---------		
3		0	1.248	2.48	0.092	1.65
3		0	1.308	2.31	0.093	1.51
3	P1	20	1.640	2.41	0.115	1.34
3	P1	40	1.232	2.15	0.147	1.30
3	P1	80	1.655	2.08	0.176	1.29
3	P2	20	1.117	2.40	0.137	1.54
3	P2	40	1.746	2.35	0.133	1.29
3	P2	80	1.715	2.18	0.150	1.13
3	P3	20	1.287	2.29	0.119	1.63
3	P3	40	1.647	2.15	0.138	1.23
3	P3	80	1.822	2.00	0.180	1.44
3	TMP	20	1.484	2.45	0.139	1.38
3	TMP	40	1.707	2.35	0.127	1.11
3	TMP	80	1.742	2.14	0.176	1.07
3	P15	20	1.352	2.41	0.123	1.41
3	P15	40	1.440	2.32	0.159	1.28
3	P15	80	1.934	2.03	0.157	1.17
3	P45	20	1.373	2.38	0.092	1.61
3	P45	40	1.605	2.25	0.119	1.25
3	P45	80	1.614	2.01	0.167	1.19

Table 47. Effect of sources and levels of P on dry matter yield, and N, P, and K content of corn grown on four soils in a pot experiment; Webster soil

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot		%-	
1		0	1.190	1.75	0.098	1.080
1		0	1.109	1.70	0.108	1.110
1	P1	20	1.205	1.57	0.140	1.144
1	P1	40	1.332	1.51	0.187	1.080
1	P1	80	1.514	1.43	0.197	1.190
1	P2	20	1.440	1.47	0.139	1.340
1	P2	40	1.464	1.41	0.144	1.120
1	P2	80	1.543	1.21	0.204	1.150
1	P3	20	1.321	1.57	0.132	1.110
1	P3	40	1.353	1.55	0.145	1.150
1	P3	80	1.420	1.51	0.238	1.100
1	TMP	20	1.163	1.57	0.139	1.250
1	TMP	40	1.464	1.56	0.164	1.220
1	TMP	80	1.471	1.33	0.218	1.200
1	P15	20	1.258	1.70	0.138	1.200
1	P15	40	1.274	1.54	0.205	1.120
1	P15	80	1.366	1.37	0.222	1.050
1	P45	20	1.423	1.59	0.128	1.100
1	P45	40	1.478	1.62	0.132	1.13
1	P45	80	1.570	1.29	0.205	1.33
2		0	1.098	1.70	0.095	1.19
2		0	1.132	1.34	0.110	1.12
2	P1	20	1.293	1.61	0.135	1.07
2	P1	40	1.521	1.57	0.151	0.88
2	P1	80	1.540	1.48	0.211	1.04
2	P2	20	1.390	1.43	0.133	1.24
2	P2	40	1.460	1.36	0.134	0.92
2	P2	80	1.615	1.24	0.178	1.06
2	P3	20	1.390	1.60	0.114	1.05
2	P3	40	1.313	1.58	0.142	1.08
2	P3	80	1.500	1.52	0.224	1.21
2	TMP	20	1.519	1.61	0.138	1.37
2	TMP	40	1.752	1.55	0.139	1.16
2	TMP	80	1.507	1.45	0.188	1.35
2	P15	20	1.171	1.60	0.132	1.26
2	P15	40	1.309	1.57	0.172	1.25
2	P15	80	1.499	1.49	0.215	1.22
2	P45	20	1.315	1.60	0.139	1.44
2	P45	40	1.447	1.58	0.146	0.93
2	P45	80	1.500	1.53	0.209	0.95

Table 47. (Continued)

Rep	P compound	Rate of P application	Dry matter yield	N	P	K
		mg/kg soil	g/pot	---------%	---------	
3		0	1.200	1.73	0.103	1.07
3		0	1.402	1.68	0.128	1.02
3	P1	20	1.162	1.45	0.159	1.20
3	P1	40	1.316	1.48	0.189	1.24
3	P1	80	1.411	1.41	0.248	1.27
3	P2	20	1.139	1.45	0.148	1.10
3	P2	40	1.446	1.40	0.175	1.12
3	P2	80	1.501	1.30	0.203	1.15
3	P3	20	1.300	1.60	0.121	1.09
3	P3	40	1.319	1.51	0.214	1.19
3	P3	80	1.488	1.48	0.225	1.29
3	TMP	20	1.322	1.65	0.151	1.13
3	TMP	40	1.531	1.42	0.144	1.13
3	TMP	80	1.552	1.54	0.198	1.31
3	P15	20	1.334	1.65	0.144	1.04
3	P15	40	1.505	1.58	0.178	1.09
3	P15	80	1.533	1.45	0.223	1.11
3	P45	20	1.434	1.61	0.123	1.06
3	P45	40	1.490	1.55	0.161	1.29
3	P45	80	1.607	1.45	0.235	1.31